



Association of *PIT1* gene polymorphism with milk productivity and body conformation traits in dairy cattle

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Marker-assisted selection (MAS) is an effective approach to accelerating genetic progress in livestock by identifying polymorphisms associated with economically important traits. The pituitary-specific transcription factor gene *PIT1* plays a regulatory role in the synthesis of growth hormone and prolactin, thus potentially influencing milk yield and body conformation in dairy cattle. This study aimed to investigate the distribution of *PIT1* genotypes and their associations with productive and morphometric traits in Ukrainian Red-and-White dairy cows – a local breed with limited genetic characterization. Genotyping was performed using PCR-RFLP with restriction enzyme *HinfI* on DNA samples obtained from 51 cows. Three genotypes (AA, AB, BB) were identified with frequencies of 0.059, 0.549, and 0.392, respectively. The population was in Hardy–Weinberg equilibrium, and the Polymorphic Information Content (PIC) value (0.34) confirmed an adequate level of variability for association analysis. The frequency of allele B was twice that of allele A, suggesting moderate genetic consolidation of the herd. Cows with the *PIT1* BB genotype showed a significantly higher milk yield per lactation (higher by 14.8%) and a 9.8% higher 305 day yield compared with the AB group. In contrast, genotype AB was associated with greater chest width (by 3.4%), as well as higher pelvic–thoracic and thoracic indexes, indicating a more robust body type. No significant differences were found among genotypes for milk fat content, udder morphology, milking speed, live weight, or service period duration. The obtained results demonstrate that *PIT1* polymorphism affects milk yield and certain conformation indexes in Ukrainian Red-and-White cattle, primarily through mechanisms related to endocrine regulation rather than skeletal development. The presence of both alleles at moderate frequencies highlights the genetic diversity of this local breed and supports its potential use in further genomic selection programs aimed at improving milk productivity while maintaining adaptive traits.

Keywords: pituitary-specific positive transcription factor 1 gene; dairy cattle; Ukrainian Red-and-White dairy breed; milk yield; body conformation; marker-assisted selection; genetic diversity.

Introduction

The application of marker-assisted selection (MAS) in animal breeding enables a substantial acceleration of genetic progress, particularly when improving traits with low heritability or when the selection differential between the best individuals of the herd and its average productivity is relatively small (Wakchaure et al., 2015; Xu et al., 2020; Das et al., 2021). Increasing productivity, in turn, is important not only for enhancing the economic efficiency of livestock production but also for reducing the negative impact of agriculture on greenhouse gas emissions – one of the major global challenges of modern times (Zos-Kior et al., 2020; Brockova et al., 2021; Zos-Kior et al., 2021). Moreover, improving animal productivity contributes to more efficient land use (Goncharov et al., 2013; Zos-Kior et al., 2016; Bilan et al., 2017). MAS is based on identifying correlations between quantitative trait loci (QTLs) and performance traits. Once markers associated with QTLs have been identified, they can be integrated into the breeding process. MAS can be applied to enhance desirable characteristics of livestock and to facilitate the utilization of existing genetic diversity within breeding populations (Ma et al., 2021; Sukhno et al., 2022; Zhukorskyi et al., 2022). The rate of genetic gain can be increased only through continuous identification of new QTLs. However, the longer selection is carried out for a particular QTL, the smaller the additional genetic improvement achieved through MAS becomes. The efficiency of MAS is greatest when productive traits are evaluated after selection for specific QTLs, allowing the selection response to be maximized (Wakchaure et al., 2015; Vashchenko et al., 2022; Saienko et al., 2023).

Genomic selection, which has evolved from MAS, is considered one of the seven most significant advances in breeding methods – both in crop science and animal husbandry – alongside transgenic animals, association mapping, envirotyping, QTL mapping, phenomics, and genome editing (Bernardo, 2016; Xu et al., 2020; Sharma et al., 2024).

According to numerous studies conducted over several decades (Machlin, 1973; Sabour et al., 1997; Saleh et al., 2024), milk productivity in cows of different breeds is influenced, among other factors, by the growth hormone (GH) gene. Since the expression of this gene is regulated by the pituitary-specific transcription factor *PIT1*, the study of *PIT1* genotype distribution and its association with productive traits is of considerable practical interest. Research by Chernenko & Hubarenko (2014) demonstrated that combinations of genotypes LL (GH) with AB or BB (*PIT1*) result in significantly higher milk yield and milk fat and protein output compared with other genotype combinations ($P < 0.001$), confirming the selective value of *PIT1* as a marker gene.

Previous studies have shown that growth hormone deficiencies in humans and mice are associated with *PIT1* mutations. As a member of the POU family of homeo-domain transcription factors, *PIT1* plays a key role in pituitary cell differentiation and proliferation, as well as in the activation of genes responsible for the synthesis of growth hormone, thyrotropin, and prolactin (PRL). Inhibition of *PIT1* expression leads to a significant reduction in PRL and GH synthesis and a drastic decrease in the proliferation of cell lines producing these hormones (Renaville et al., 1997; Alzaydi & Salim, 2021).

At present, *PIT1* gene polymorphism in cattle has been well characterized for many breeds. According to the review by Ebrahimi

Hoseinzadeh et al. (2015), the allele ratio of PIT1 in European dairy breeds such as Holstein, Holstein-Friesian, and other related Black-and-White breeds ranges from 0.16:0.84 to 0.28:0.32 (A:B). In contrast, beef breeds such as Angus and Belgian Blue demonstrate a substantially higher frequency of the A allele – 0.33 and 0.42, respectively (Ebrahimi Hoseinzadeh et al., 2015). However, no data were found in the available literature regarding the genotype distribution of this gene in the Ukrainian Red-and-White dairy breed (URWD), which is the second most numerous breed in Ukraine.

The distribution of genotype frequencies within a marker locus is essential for assessing the feasibility of further association studies, since a population must exhibit sufficient genetic variability to demonstrate correlations with productive traits. According to Botstein et al. (1980), for a valid association analysis, the Polymorphism Information Content (PIC) value should range between 0.25 and 0.75. Considering that local breeds – including the URWD – may exhibit unique allele frequency patterns (Giovambattista et al., 2001; Hohmann et al., 2021; Eiriksson et al., 2022), the study of the PIT1 polymorphism in such populations is important not only for Ukraine but also in the broader global context of preserving livestock genetic diversity.

Materials and methods

In conducting the research, strict adherence was maintained to the Order of the First National Congress on Bioethics (Kyiv, 2001), as well as the Law of Ukraine “On the Protection of Animals from Cruelty” No. 3447-IV of 21 February 2006, with subsequent amendments as of 4 August 2017. The experimental protocol was formally approved by the Committee for the Maintenance and Use of Animals at the Poltava State Agrarian University. All procedures were carried out with a strong emphasis on animal welfare. The cows were kept in a free-stall housing system, which allowed them to express their natural physiological and behavioral needs with minimal restrictions. During the study, blood samples were collected for genetic analysis using procedures designed to minimize discomfort and stress. The health status and overall condition of the animals were carefully monitored throughout the experimental period to ensure compliance with welfare standards. In addition, the research team followed the 3Rs principle (Replacement, Reduction, Refinement) established in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1985). This approach minimized potential stress, improved sampling accuracy, and prevented unnecessary procedures. Consequently, the study not only met ethical standards but also strengthened the reliability and validity of the obtained results.

The experimental animals were cows of the Ukrainian Red-and-White dairy breed belonging to the private agricultural company “Ukraina”, located in the Myrhorod district of the Poltava region (Ukraine). The study focused on identifying associations between the animals’ genotypes for the pituitary-specific transcription factor gene PIT1 and their milk productivity traits within this local breed. The following traits were recorded for each animal: milk yield for 305 days of lactation (kg), total milk yield per lactation (kg), milk ejection rate (kg per minute). The following body measurements were recorded for each cow: height at withers (cm) – measured vertically from the ground to the highest point of the withers; oblique body length (cm) – measured from the anterior edge of the shoulder joint to the ischial tuberosity; chest depth (cm) – measured vertically from the highest point of the withers to the lowest point of the sternum; chest width (cm) – measured as the distance between the lateral surfaces of the shoulder joints; chest girth behind the shoulder blades (cm) – measured with a measuring tape around the thorax immediately behind the shoulder blades; cannon bone girth (cm) – measured at the middle of the metacarpus; live weight (kg) – determined using livestock scales immediately after the morning milking and before feeding. Body measurements were taken using a measuring stick with a scale division value of ± 1 mm (Nasco Education, Ajax, Canada) and a measuring tape (Kruuse, Langeskov, Denmark). The animals were weighed on electronic scales with a measurement accuracy of 0.5 kg (Dnipro, Ukraine).

To assess the proportional development of the body and the exterior type of cows, the following body conformation indexes were calculated based on linear body measurements: the leg length index was determined as the ratio of the height at the withers to the oblique body length, expressed as a percentage; the body length index was calculated as the ratio of the oblique body length to the height at the withers; the pelvic-thoracic index was defined as the ratio between the width of the rump (hip width) and the width of the chest; the thoracic index was obtained by comparing the chest girth measured behind the shoulder blades with the height at the withers; the compactness index was calculated as the ratio of chest girth to oblique body length; the height proportion index was determined by dividing the height at the withers by the oblique body length. It indicates the proportionality between the animal’s height and body length, showing whether the cow has a tall or elongated body type; the boneiness index was estimated as the ratio of the cannon bone girth to the height at the withers. To assess reproductive performance, the length of the service period (days) was also calculated.

The experimental work was carried out in several consecutive stages (Fig. 1).

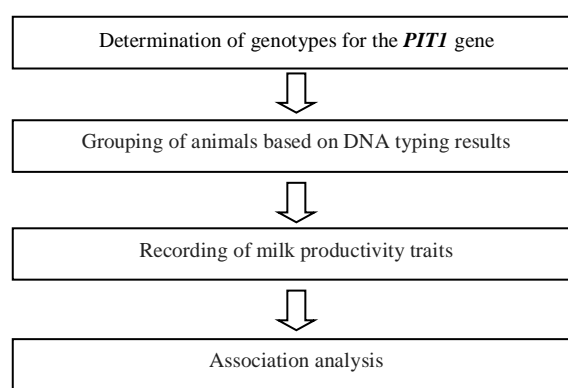


Fig. 1. Research organization scheme

During the experimental period, all cows were provided with a feeding ration balanced according to zootechnical standards (Ibatullin et al., 2016). The nutrient composition of the diets was adjusted based on the average live weight of 550 kg and the mean daily milk yield, which varied throughout the 305-day lactation period from 26.5 kg in the second month to 15.1 kg in the tenth month. Because the feeding standards for dairy cows are presented in discrete increments of 5 kg of daily milk yield, three ration variants were used in the experiment – balanced for 15, 20, and 25 kg of daily milk yield, respectively.

According to their main nutritional characteristics, these rations contained, respectively, 156.6, 177.5, and 202.8 MJ of metabolizable energy; 2759, 2836, and 3120 g of crude protein; 2346, 2269, and 2340 g of rumen-degradable protein; 413, 567, and 780 g of rumen-undegradable protein; 1780, 1830, and 2047 g of digestible protein; 4272, 4392, and 3900 g of crude fiber; 2403, 2470, and 3120 g of starch; 1602, 1647, and 2047 g of sugars; 570, 586, and 702 g of crude fat; 115.7, 119.0, and 126.8 g of calcium; and 80.1, 82.3, and 87.8 g of phosphorus.

In the winter period, the ration included sainfoin hay, grass hay, alfalfa hay, silage, fodder beet, and concentrate feeds. To meet mineral and vitamin requirements, a 4% protein-mineral-vitamin supplement was added to the diet. During the summer period, the cows were fed green maize at the milk-wax stage of maturity, fresh alfalfa, Sudan grass and oat hay, barley straw, and the same protein-mineral-vitamin supplement.

Cows were kept under a loose-housing system in well-ventilated barns that met zoohygienic standards. Milking was carried out in a milking parlor equipped with a modern digital monitoring system, which enabled automatic animal identification and recording of individual milk yields and milk flow rate (Fig. 2).

Molecular-genetic analyses were performed at the certified Laboratory of Genetics of the Research Institute of Pig Breeding and Agro-Industrial Production, which holds Certificate of Accreditation

No. 039-22 dated June 3, 2022, issued by the Ministry of Economic Development and Trade of Ukraine (State Enterprise “Poltava Regional Scientific and Technical Center for Standardization, Metrology and Certification”).

Genomic DNA was isolated from 200 μ L of blood using the Chelex 100 (Bio-Rad Laboratories, Inc., USA) method (Walsh et al., 2013). DNA genotyping was conducted using the PCR-RFLP technique (Hlazko et al., 2001). The reaction was performed in a Tercyk-2 thermocycler (Tersik TP4-PCR01, DNA-Technology, RF) using 0.5 mL Eppendorf microcentrifuge tubes (Eppendorf, Germany) with a total volume of 25 μ L. The optimized conditions for PCR amplification of the PIT1 gene fragment included an annealing temperature of 55 $^{\circ}$ C, allowing the synthesis of an expected product with a length of 1355 base pairs. The reaction protocol comprised an initial denatura-

tion at 94 $^{\circ}$ C for 3 minutes, followed by 35 amplification cycles. Each cycle consisted of denaturation at 94 $^{\circ}$ C for 30 seconds, primer annealing at 55 $^{\circ}$ C for 35 seconds, and elongation at 72 $^{\circ}$ C for 45 seconds. The reaction was completed by a final elongation step at 72 $^{\circ}$ C for 2 minutes.

The specific primers used for amplification were as follows:

Forward: 5'-CAATGAGAAAGTTGGTGC-3'

Reverse: 5'-TCTGCATTCGAGATGCTC-3'

The polymorphism of the PIT1 gene selected for the study contains a recognition site for the HinfI restriction endonuclease (Thermo Fisher Scientific, Lithuania). As a result of HinfI digestion, DNA fragments of the following lengths were expected: allele A – 660 bp, 425 bp, and 270 bp; and allele B – 660 bp, 385 bp, 270 bp, and 40 bp.

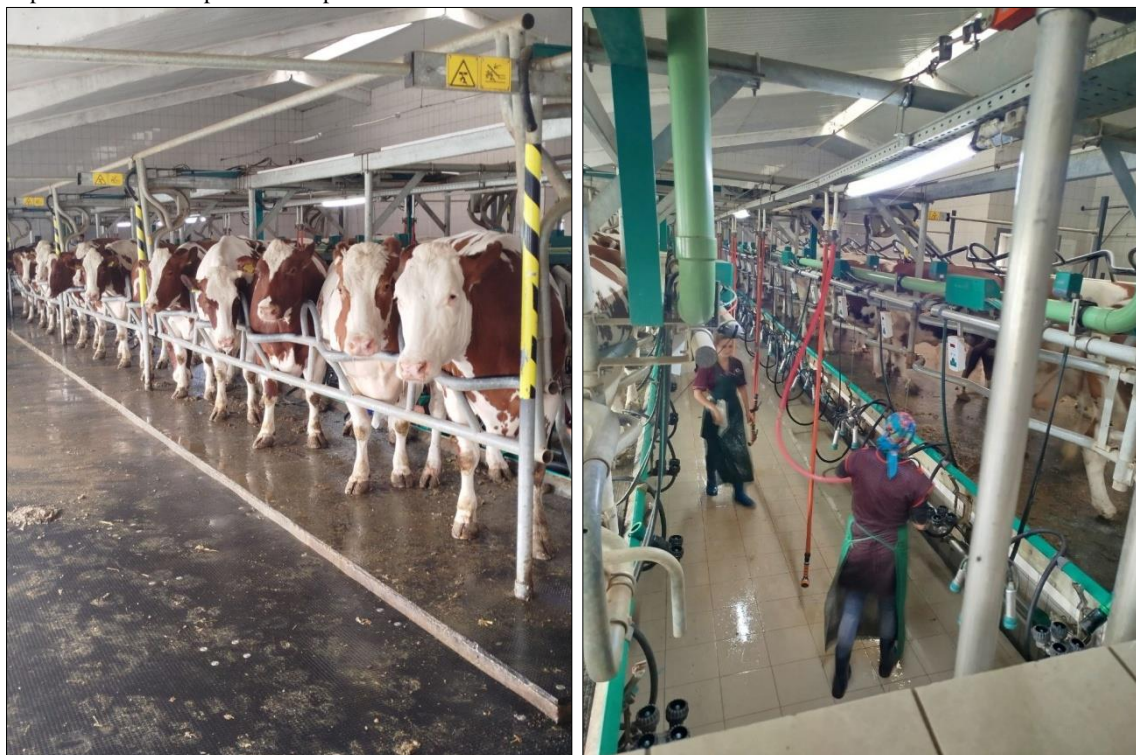


Fig. 2. Cows of the Ukrainian Red-and-White dairy breed in the milking parlor (private agricultural company “Ukraina”, Poltava Region)

Electrophoretic analysis of the amplification products was performed in a 2% agarose gel under a current of 76 mA and a voltage of 275 V. Visualization was carried out by staining the gel with ethidium bromide, followed by examination under ultraviolet light using a transilluminator. According to the electrophoresis results, the sizes of the obtained DNA fragments corresponded to the expected ones relative to the molecular weight marker DNA 1 Kb Ladder (Fig. 3).

The GenAlEx 6.0 software package (Peakall, Australia, 2012) was used to calculate allele and genotype frequencies and to determine the polymorphism information content (PIC) (Peakall, 2012). The Chi-square test was applied to assess the consistency between observed and expected genotype frequencies.

Statistical processing of productive and morphological traits was performed using SAS/STAT[®] 15.1 (SAS Institute Inc., USA, 2018). The tables present the arithmetic means and their standard errors ($\bar{x} \pm SE$). Differences between groups were evaluated using one-way analysis of variance (ANOVA). The ratio of intergroup to intragroup variability was estimated with Fisher’s F-test. Multiple comparisons were performed using Tukey’s HSD test, and differences were considered statistically significant at $P < 0.05$.

Results

As a result of carrying out laboratory genetic and molecular studies, the genotypes of experimental cows were determined according to the pituitary-specific transcription factor (PIT1) gene. Distribution of

genotypes was: AA – 3 animals, AB – 28 animals, BB – 20 animals (Table 1).

It was established that the frequency of allele B was approximately twice as high as that of allele A. Analysis of the genotype distribution showed that animals carrying the heterozygous genotype AB predominated in the population. In particular, this genotype occurred 9.3 times more frequently than genotype AA and 1.4 times more frequently than genotype BB. At the same time, genotype BB was found 6.6 times more often than genotype AA.

A comparison of the observed and expected genotype distributions revealed no statistically significant differences, as confirmed by the Chi-square test. This indicates that the studied population does not deviate significantly from Hardy–Weinberg equilibrium.

The level of polymorphism at the PIT1 locus was sufficient for conducting association studies. The Polymorphism Information Content (PIC) was 0.34, which falls within the optimal range for such analysis and provides adequate genotype variability to detect potential relationships with productive traits. The observed and expected heterozygosity values for the studied locus were $H_o = 0.549$ and $H_e = 0.444$, respectively, which also correspond to optimal parameters (Fig. 4). In the cattle population genotyped at the PIT1 locus ($n = 51$), two distinct alleles ($N_a = 2$) were identified, both occurring at frequencies above 5% ($N_a \text{ Freq.} \geq 5\% = 2.000$). The number of effective alleles (N_e), reflecting the actual genetic diversity, was 1.800. The Shannon information index (I), which accounts for both the number of alleles and the evenness of their frequencies, was 0.637, indicating

a relatively low but still detectable level of allelic diversity. Two private alleles (No. Private Alleles = 2.000) characteristic of this population were recorded. At the same time, locally common alleles, defined

as those occurring in $\leq 25\%$ or $\leq 50\%$ of populations (No. LComm Alleles), were absent in this sample.

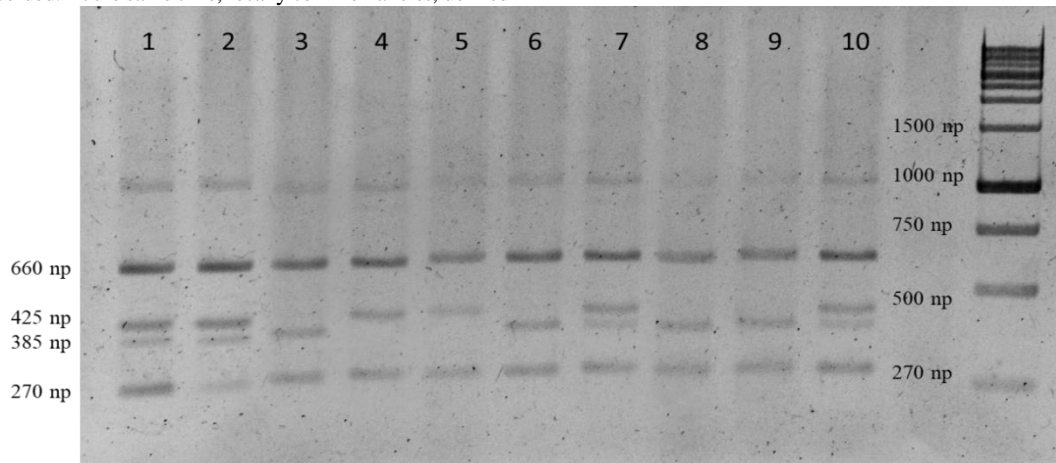


Fig. 3. Electropherogram of the *PIT1* gene in 2% agarose gel. lanes 1, 2, 7, and 10 – genotype AB; lanes 4 and 5 – genotype AA; lanes 3, 6, 8, and 9 – genotype BB; M – molecular weight marker 1 Kb DNA Ladder

Table 1
Allelic and genotypic frequency distribution of the *PIT1* gene in cows (n = 51)

Locus	Allele frequencies		Genotype frequencies			χ^2	P	F
	A	B	AA	AB	BB			
<i>PIT1</i>	0.333	0.667	0.059 (0.11)	0.549 (0.45)	0.392 (0.44)	2.824	0.093	0.235

Note: values in parentheses represent the expected genotype distribution.

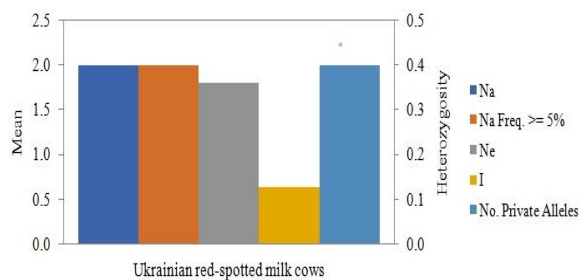


Fig. 4. Allelic patterns across populations

The expected heterozygosity (H_e) was 0.444, while the unbiased expected heterozygosity (uH_e), adjusted for the limited sample size, was 0.449. These results indicate a moderate level of heterozygosity within the population. Thus, the cattle population under study at the *PIT1* locus is characterized by a small number of alleles but also the presence of private alleles, which highlights its genetic specificity. The obtained heterozygosity and information index values demonstrate the preservation of a certain degree of within-population diver

sity, although overall diversity remains limited. The moderate level of heterozygosity suggests partial genetic homogeneity, and the absence of significant deviation from Hardy–Weinberg equilibrium confirms that no detectable effects of inbreeding or selection were observed at this locus.

An association analysis was performed to evaluate the relationships between *PIT1* genotypes and milk productivity traits, live weight, and selected linear body measurements (Table 2). According to the results of Tukey's post hoc test, a statistically significant difference was observed for three traits only between groups AB and BB, whereas the parameters of cows with genotype AA did not differ significantly from either of the other two groups. In particular, cows carrying the AB genotype produced 1,017.3 kg less milk per lactation than cows with the BB genotype, which is 14.8% lower compared with the BB group. For the 305-day milk yield, the difference between these two groups amounted to 706.2 kg, or 9.8%, in favor of the BB genotype.

Regarding chest width, cows with the *PIT1* AB genotype exceeded those with the *PIT1* BB genotype by 1.89 cm, indicating that AB cows had a 3.4% wider chest region.

Table 2
Effect of *PIT1* genotype on milk productivity, live weight, and linear body measurements of Ukrainian Red-and-white cows ($\bar{x} \pm SE$)

Productive traits	Genotype			F	P
	AA, n = 3	AB, n = 28	BB, n = 20		
Milk yield per lactation, kg	8149.3 \pm 1698.3 ^{ab}	6872.4 \pm 195.5 ^a	7889.7 \pm 382.2 ^b	3.334	4.41*10 ⁻²
Milk yield for 305 days, kg	7346.0 \pm 985.2 ^{ab}	6521.6 \pm 156.1 ^a	7227.8 \pm 165.1 ^b	4.586	1.51*10 ⁻²
Height at withers, cm	141.00 \pm 7.57	138.75 \pm 1.10	139.50 \pm 1.17	0.232	0.794
Oblique body length, cm	39.67 \pm 0.33	38.93 \pm 0.24	39.20 \pm 0.22	0.278	0.758
Chest depth, cm	81.00 \pm 3.06	79.07 \pm 0.84	80.20 \pm 0.97	0.527	0.594
Chest width, cm	52.33 \pm 1.20 ^{ab}	55.04 \pm 0.52 ^a	53.15 \pm 0.53 ^b	3.912	2.67*10 ⁻²
Chest girth behind the shoulder blades, cm	203.67 \pm 9.13	201.50 \pm 1.14	202.90 \pm 1.66	0.284	0.754
Cannon bone girth, cm	19.33 \pm 0.33	19.64 \pm 0.11	19.70 \pm 0.13	0.552	0.579
Live weight, kg	510.00 \pm 28.87	516.07 \pm 4.79	519.00 \pm 7.40	0.139	0.870

Note: different letters within each row indicate significant differences between groups according to Tukey's HSD test results.

For other morphometric traits – including height at withers, oblique body length, chest depth, chest girth behind the shoulder blades, cannon bone girth, and live weight – no statistically significant differences were detected among genotype groups. Cows with the

AA genotype were taller, with an elongated and narrower chest, whereas heterozygous AB animals were shorter but had a broader body conformation. Although linear body measurements reflect the absolute dimensions of the animals, a more informative indicator of

conformation is the relationship among these measurements, expressed as body conformation indexes. These indexes were calculated to provide a comprehensive assessment of exterior type (Table 3).

No statistically significant differences were found among the *PIT1* genotypes for most body conformation indexes, including leg length, body length, compactness, height proportion, and boneiness. However, a significant effect of the *PIT1* genotype was established for the pelvic-thoracic and thoracic indexes. For the former, cows with the AB genotype exceeded those with the AA genotype by

9.55 units (7.24%) and those with the BB genotype by 5.83 units (4.30%). Regarding the thoracic index, AB cows also showed a tendency toward higher values compared with the other groups, although a statistically significant difference was observed only between the AB and BB groups, amounting to 3.34 units, or 5.02%. These findings indicate that polymorphism in the *PIT1* gene primarily influences the proportional development of the thoracic and pelvic regions of the body, whereas other exterior indexes remain relatively stable regardless of genotype.

Table 3

Effect of *PIT1* genotype on body conformation indexes of Ukrainian Red-and-White cows ($x \pm SE$)

Body composition index	Genotype			F	P
	AA	AB	BB		
Leg length, units	42.41 \pm 1.84	43.00 \pm 0.47	42.51 \pm 0.50	0.273	0.762
Body length, units	121.25 \pm 4.19	122.72 \pm 0.79	122.85 \pm 0.75	0.206	0.815
Pelvic-thoracic, units	131.90 \pm 1.98 ^a	141.45 \pm 1.33 ^b	135.62 \pm 1.31 ^a	6.410	3.41*10 ⁻³
Thoracic, units	64.90 \pm 3.82 ^{ab}	69.77 \pm 0.87 ^a	66.43 \pm 0.96 ^b	3.893	2.71*10 ⁻²
Compactness, units	119.46 \pm 3.01	118.52 \pm 0.74	118.50 \pm 0.83	0.083	0.921
Height proportion, units	103.38 \pm 5.06	104.42 \pm 0.92	103.99 \pm 0.79	0.096	0.908
Boniness, units	13.77 \pm 0.53	14.18 \pm 0.14	14.14 \pm 0.16	0.384	0.683

Note: different letters within each row indicate significant differences between groups according to Tukey's HSD test results.

When assessing udder characteristics, it was found that all cows under study had a cup-shaped udder, and the average score for udder conformation did not differ significantly among genotypic groups, averaging 36.01 \pm 0.11 points across the sample. The *PIT1* genotype also had no significant effect on milk flow rate, which averaged 1.9062 \pm 0.0105 kg/min.

Evaluation of the effect of different genotypes on the duration of the service period also revealed no statistically significant differences among the groups (F = 1.3424; P = 0.2709). Although the mean value of this trait in cows with the AA genotype (X = 219.0 days) was 1.68 times higher than that in cows with the BB genotype (X = 30.75 days), the trait exhibited very high variability (standard errors were 185.98 and 69.45 days, respectively).

Discussion

The results of the population-genetic analysis indicate a moderate level of genetic variability within the studied sample. The presence of only two alleles with an uneven distribution suggests a certain degree of genetic consolidation in the herd, which is typical of populations subjected to long-term selection for productive traits. The observed balance between heterozygosity parameters (Ho and He), as well as the absence of deviation from the Hardy-Weinberg equilibrium, confirms the stability of allele frequencies and the lack of strong selection pressure at the *PIT1* locus (Selvaggi & Dario, 2011).

Allele B was found to be twice as common in the population of Ukrainian Red-and-White dairy cattle as allele A. Similar allele distributions were reported in young bulls of the Podolica breed (Selvaggi et al., 2011), where the frequency of allele A was 30.0%, being 2.33 times lower than that of allele B. In contrast, in Holstein cows (Renaville et al., 1997), the predominance of allele B over A was even greater – by a factor of 4.32, with respective frequencies of 18.8% and 81.2%. When comparing the genotype frequencies of the *PIT1* gene identified in the Ukrainian Red-and-White breed with those reported for the Holstein breed (Renaville et al., 1997), it was observed that Holsteins had a higher proportion of BB homozygotes (66.3%), whereas the frequencies of AA and AB genotypes were markedly lower (2.2% and 31.5%, respectively). In other studies involving Holstein and Simmental cattle, the predominance of allele B was also confirmed, with its frequency reaching up to 90.0% (Aytekin & Fadhil, 2022). Previous research associated this allele with improved milk productivity, while allele A was more closely linked to certain body conformation traits.

Considering that the Ukrainian Red-and-White dairy breed was developed using Simmental cattle as the maternal base, which were then crossed with Holstein and Ayrshire sires (Kruhliak et al., 2015; Machulnyi, 2018), such an allele distribution is entirely expected. Therefore, our results are consistent with general genetic patterns ob-

served in dairy cattle populations. The *PIT1* gene encodes a pituitary-specific transcription factor that regulates the expression of genes responsible for the synthesis of growth hormone and prolactin (Cohen et al., 1996; Alzaydi & Salim, 2021). The pattern observed in our study – higher milk productivity in cows with the BB genotype compared with AB heterozygotes – aligns with the findings of a recent meta-analysis by Çelik Gürbulak & Aksel (2025), which indicated that for the *Pit1-HinfI* polymorphism, the BB genotype and allele B are the most prevalent. That analysis demonstrated that allele distributions in Holstein, Simmental, and Brown Swiss cattle are shaped by productivity level, whereas local breeds tend to maintain greater genetic diversity. The authors also associated allele B with higher milk yield, while allele A was characterized as a significant indicator of sustainability, potentially playing an important role in preserving the genetic background in the future.

However, our findings contrast with those of studies on Holstein cows (Renaville et al., 1997; Doosti et al., 2011), in which higher milk productivity was linked to the AA genotype. Thus, the substantial differences in milk yield among genotypes may reflect the regulatory role of this gene in lactation physiology. The lack of significant differences in most body conformation traits suggests that *PIT1* polymorphism primarily affects metabolic and hormonal pathways rather than skeletal development (Prihandini et al., 2025).

Similar conclusions were drawn by other researchers as well (Renaville et al., 1997), who applied canonical transformation of the data to increase the accuracy of analysis, which proved effective for separating the effects on interrelated traits. However, the association between *PIT1* gene polymorphism and milk productivity traits in cows was confirmed both in the raw data and after statistical transformation. Consistent with our results, no significant influence of allele A on exterior characteristics was detected, except for traits such as body depth and angularity, which are known to be correlated with milk performance (Renaville et al., 1997). Our findings support this observation, since the 3.4% narrower chest width observed in BB cows compared with AB heterozygotes is also a characteristic feature of the “dairy type” of cattle. Thus, the narrower chest in cows with higher milk productivity corresponds to the body conformation patterns previously described by other authors (Renaville et al., 1997).

Because the transcription factor *PIT1* participates in regulating the expression of several anterior pituitary genes – particularly those controlling the synthesis and secretion of hormones involved in lactation – its indirect effect on milk ejection can be hypothesized. Specifically, *PIT1* has been reported to interact with other regulatory proteins, such as C/EBP α , in the control of oxytocin (OXT) gene transcription, which directly mediates myoepithelial cell contraction and milk release (Pauciullo et al., 2012). Based on this, we proposed the hypothesis that *PIT1* gene polymorphisms may influence milk ejection rate through alterations in oxytocin system activity. To test this hy-

pothesis, the milk flow rate was measured in the studied cows, and an association analysis was performed with *PIT1* genotypes. However, no statistically significant relationship between genotype and milk flow rate was detected.

Furthermore, both our data and previously published studies revealed no significant association between *PIT1* polymorphism and the duration of the days open period, which indirectly characterizes the reproductive ability of cows (Temesgen et al., 2022).

The results obtained indicate that *PIT1* gene polymorphism may serve as a potential genetic marker of milk productivity in Ukrainian Red-and-White cattle. However, to confirm the observed tendencies and to determine the combined effect of multiple loci on productive and morphological traits, further studies involving larger datasets and additional candidate genes are required.

Conclusions

The Ukrainian Red-and-white dairy population demonstrated two alleles (A and B) at the *PIT1* locus with moderate polymorphism (PIC = 0.34) and no deviation from the Hardy-Weinberg equilibrium. The B allele predominated in the population, occurring with twice the frequency of allele A, indicating genetic similarity to European dairy breeds. Cows with the *PIT1* BB genotype had higher milk yield per lactation and per 305 days compared to heterozygous AB individuals ($P < 0.05$). Genotype AB was associated with larger chest width and higher pelvic-thoracic and thoracic indexes, reflecting a more developed body frame. The *PIT1* polymorphism did not significantly affect udder morphology, milking speed, or reproductive traits (service period duration).

The results confirm the potential of the *PIT1* gene as a molecular marker for milk productivity traits in Ukrainian Red-and-White dairy cattle and justify further multi-locus genomic studies to improve the breed's selection efficiency.

The authors declare no conflicts of interest.

References

Alzaydi, M. K. M., & Salim, A. H. (2021). Detection of the effect of PIT-1 gene genotypes on some productive traits in Holstein cattle. *University of Thi-Qar Journal of Agricultural Research*, 10(2), 8–15.

Aytekin, I., & Fadhil, M. (2022). Pit-1 gene polymorphisms in Anatolian Black, Holstein Friesian, Brown Swiss and Simmental cattle reared in Turkey. *Journal of Agricultural Sciences*, 28(1), 139–144.

Bernardo, R. (1996). Best linear unbiased prediction of maize single-cross performance. *Crop Science*, 36(1), 50–56.

Bilan, Y., Zos-Kior, M., Nitsenko, V., Sinelnikau, U., Ilin, V. (2017). Projecting the social component of the efficient management of land resources. *Journal of Security and Sustainability Issues*, 7(2), 287–300.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, 32(3), 314–331.

Brockova, K., Rossokha, V., Chaban, V., Zos-Kior, M., Hnatenko, I., & Rubczanska, V. (2021). Economic mechanism of optimizing the innovation investment program of the development of agro-industrial production. *Management Theory and Studies for Rural Business and Infrastructure Development*, 43(1), 129–135.

Çelik Gürbulak, E., & Aksel, E. G. (2025). Proportion of Pit-1 gene *Hinf*I polymorphism in different cattle breeds: A meta-analysis. *Acta Veterinaria*, 75(3), 29.

Chemenko, O., & Hubarenko, N. (2014). Vplyv henotypu za henamy GH ta PIT-1 na molochnist holshtynskykh koriv [The influence of genotype for GH and PIT-1 genes on milk yield of Holstein cows]. *Tvarynymystvo Ukrainy*, 11, 31–35 (in Ukrainian).

Cohen, L. E., Wondisford, F. E., & Radovick, S. (1996). Role of Pit-1 in the gene expression of growth hormone, prolactin, and thyrotropin. *Endocrinology and metabolism clinics of North America*, 25(3), 523–540.

Das, D. N., Karuthadurai, T., & Gnanasekaran, S. (2021). Genomic selection: A molecular tool for genetic improvement in livestock. In: Mondal, S., & Singh, R. L. (Eds.). *Advances in animal genomics*. Academic Press, Amsterdam. Pp. 141–163.

Doosti, A., Arshi, A., & Momeni, B. (2011). Molecular study of PIT1 gene polymorphism in Holstein and Iranian native cattle. *African Journal of Agricultural Research*, 6(19), 4467–4470.

Ebrahimi Hoseinzadeh, Z., Mohammadabadi, M. R., Esmailizadeh, A. K., & Khezri, A. (2015). Association of PIT1 gene and milk protein percentage in Holstein cattle. *Journal of Livestock Science and Technologies*, 3(1), 40–49.

Eiriksson, J. H., Strandén, I., Su, G., Mäntysaari, E. A., & Christensen, O. F. (2022). Local breed proportions and local breed heterozygosity in genomic predictions for crossbred dairy cows. *Journal of Dairy Science*, 105(12), 9822–9836.

Giovambattista, G., Ripoli, M. V., Peral-Garcia, P., & Bouzat, J. L. (2001). Indigenous domestic breeds as reservoirs of genetic diversity: the Argentinean Creole cattle. *Animal Genetics*, 32(5), 240–247.

Goncharov, V. M., Zos-Kior, M., & Rakhmetulina Zhibek, B. (2013). The investment component of Ukrainian agrarian enterprises' development in conditions of land reform. *Actual Problems of Economics*, 10(148), 118–125.

Hlazko, V. Y., Shulha, E. V., Dyman, T. N., & Hlazko, H. V. (2001). DNK-tehnolohii bioinformatika v reshenii problem byotekhnologii mlekoopitaiushchykh [DNA technologies and bioinformatics in solving the problems of mammalian biotechnologies]. Belotserkovskiy Agrarian University, Belaya Tserkov (in Russian).

Hohmann, L. G., Weimann, C., Scheper, C., Erhardt, G., & König, S. (2021). Genetic diversity and population structure in divergent German cattle selection lines on the basis of milk protein polymorphisms. *Archives Animal Breeding*, 64(1), 91–102.

Ibatullin, I. I., Bashchenko, M. I., Zhukorskiy, O. M., Kandyba, V. M., Rudenko, Y. V., Ionov, I. A., Mykhalchenko, S. A., Tsvihun, A. T., Shapovalov, S. O., Zolotarov, A. P., Yeletska, L. M., Yurchenko, S. H., Kravchenko, Y. S., Saprykin, V. O., Haziiev, B. M., Kosov, M. O., Pomitun, I. A., Kosova, N. O., Tkachova, I. V., Vakulenko, I. S., Kostenko, V. I., Boiarchuk, S. V. (2016). Dovidnyk z povnotsinnoyi hodivli sil'skohospodars'kykh tvaryn [Handbook of balanced feeding of farm animals]. In: Ibatullin, I. I., & Zhukorskiy, O. M. (Eds.). *Ahrarna nauka* (in Ukrainian).

Kruhliak, A. P., Biriukova, O. D., Kovalenko, H. S., & Kruhliak, T. O. (2015). Ukrainys'ka chervono-riaba molochna poroda – rezul'tat realizatsiyi novoyi teorii u skotarstvi [Ukrainian Red-and-White dairy breed – the result of the implementation of a new theory in cattle breeding]. *Animal Breeding and Genetics*, 50, 39 (in Ukrainian).

Ma, Y., Khan, M. Z., Xiao, J., Alugongo, G. M., Chen, X., Chen, T., Liu, S., He, Z., Wang, J., & Kamal Sha, M. (2021). Genetic markers associated with milk production traits in dairy cattle. *Agriculture*, 11(10), 1018.

Machlin, L. J. (1973). Effect of growth hormone on milk production and feed utilization in dairy cows. *Journal of Dairy Science*, 56(5), 575–580.

Machulnyi, V. V. (2018). Productivity of cows of Ukrainian Black-and-White and Red-and-White dairy breeds. *Animal Breeding and Genetics*, 51, 112–118.

Paucullo, A., Cosenza, G., Steri, R., Coletta, A., Jemma, L., Feligini, M., Di Berardin, D., P. P. Macciotta, N., & Ramunno, L. (2012). An association analysis between OXT genotype and milk yield and flow in Italian Mediterranean river buffalo. *Journal of Dairy Research*, 79(2), 150–156.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics*, 28, 2537–2539.

Prihandini, P. W., Hariyono, D. N. H., Sari, A. P. Z. N. L., Tribudi, Y. A., Ibrahim, A., Luthfi, M., Wiyono, A., Irmawanti, S., Aryogi, A., Khosiya Robba, D., Chanafi, M., Kuswa, K., & Leandro, H. (2025). Association between GH, PRL, LEP, and PIT-1 gene polymorphisms and growth traits in Indonesian Rambon indigenous cattle. *Tropical Animal Health and Production*, 57(2), 56.

Renaville, R., Gengler, N., Vrech, E., Prandi, A., Massart, S., Corradini, C., Bertozzi, C., Mortiaux, F., Burny, A., & 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.

Sabour, M. P., Lin, C. Y., & Smith, C. (1997). Association of genetic variants of bovine growth hormone with milk production traits in Holstein cattle. *Journal of Animal Breeding and Genetics*, 114(1–6), 435–442.

Saienko, A., Peka, M., Tsereniuk, O., Babicz, M., Kropiwiw-Domańska, K., Onyshchenko, A., Vashchenko, P., & Balatsky, V. (2023). Analysis of polymorphism and development of a molecular-genetic system for genotyping by the telomerase reverse transcriptase (TERT) gene. *Biosystems Diversity*, 31(4), 436–443.

Saleh, A. A., Hassan, T. G., El-Hedainy, D. K., El-Barbary, A. S., Sharaby, M. A., Hafez, E. E., & Rashad, A. M. (2024). IGF-I and GH genes polymorphism and their association with milk yields, composition and reproductive performance in Holstein-Friesian dairy cattle. *BMC Veterinary Research*, 20(1), 341.

Selvaggi, M., & Dario, C. (2011). Analysis of two Pit-1 gene polymorphisms: Single nucleotide polymorphisms (SNPs) distribution patterns in Podolica cattle breed. *African Journal of Biotechnology*, 10(55), 11360–11364.

Selvaggi, M., Dario, C., Normanno, G., Dambrosio, A., & Dario, M. (2011). Analysis of two pit-1 gene polymorphisms and relationships with growth performance traits in Podolica young bulls. *Livestock Science*, 138(1–3), 308–312.

- Sharma, P., Daultani, S., Hadiya, K. K., George, L. B., & Highland, H. N. (2024). Overview of marker-assisted selection in animal breeding. *Journal of Advances in Biology and Biotechnology*, 27(5), 303–318.
- Sukhno, V. V., Vashchenko, P. A., Saenko, A. M., Zhukorskyi, O. M., Tserenyuk, O. M., & Kryhina, N. V. (2022). Association of Fut1 and Slc11a1 gene polymorphisms with productivity traits of Large White pigs. *Regulatory Mechanisms in Biosystems*, 13(3), 225–230.
- Temesgen, M. Y., Assen, A. A., Gizaw, T. T., Minalu, B. A., & Mersha, A. Y. (2022). Factors affecting calving to conception interval (days open) in dairy cows located at Dessie and Kombolcha towns, Ethiopia. *PLoS One*, 17(2), e0264029.
- Vashchenko, P., Saienko, A., Sukhno, V., Tsereniuk, O., Babicz, M., Shkavro, N., Smolucha, G., & Łuszczewska-Sierakowska, I. (2022). Association of NRAMP1 gene polymorphism with the productive traits of the Ukrainian Large White pig. *Medycyna Weterynaryjna*, 78(11), 563–566.
- Wakchaure, R., Ganguly, S., Praveen, P. K., Kumar, A., Sharma, S., & Mahajan, T. (2015). Marker assisted selection (MAS) in animal breeding: A review. *Journal of Drug Metabolism and Toxicology*, 6(5), e127.
- Walsh, P. S., Metzger, D. A., & Higuchi, R. (2013). Chelex 100 as a medium for extraction of DNA for PCR-based typing from forensic material. *Bio-techniques*, 54(3), 134–139.
- Xu, Y., Liu, X., Fu, J., Wang, H., Wang, J., Huang, C., Prasanna, B. M., Olsen, M. S., Wang, G., & Zhang, A. (2019). Enhancing genetic gain through genomic selection: From livestock to plants. *Plant Communications*, 1(1), 100005.
- Zhukorskyi, O. M., Tsereniuk, O. M., Vashchenko, P. A., Khokhlov, A. M., Chereuta, Y. V., Akimov, O. V., & Kryhina, N. V. (2022). The effect of the ryanodine receptor gene on the reproductive traits of Welsh sows. *Regulatory Mechanisms in Biosystems*, 13(4), 367–372.
- Zos-Kior, M., Hnatenko, I., Isai, O., Shtuler, I., Samborskyi, O., & Rubezhanska, V. (2020). Management of efficiency of the energy and resource saving innovative projects at the processing enterprises. *Management Theory and Studies for Rural Business and Infrastructure Development*, 42(4), 504–515.
- Zos-Kior, M., Kuksa, I., Ilyin, V., Chaikina, A. (2016). Land management prospects. *Economic Annals-XXI*, 9–10, 243–246.
- Zos-Kior, M., Shkurupii, O., Fedirets, O., Shulzhenko, I., & Rubezhanska, V. (2021). Modeling of the investment program formation process of ecological management of the agrarian cluster. *European Journal of Sustainable Development*, 10(1), 571–583.