



Polymorphism of the *ADRB3* gene in Ukrainian Red-and-White Dairy cattle and its association with economically important traits

P. A. Vashchenko*, V. A. Polishchuk*, O. M. Tsereniuk**, I. A. Balabanova***, O. I. Stadnytska****, B. S. Shaferivskyi*, O. G. Fesenko*, A. A. Polishchuk*

* *Poltava State Agrarian University, Poltava, Ukraine*

** *Institute of Pig Breeding and Agroindustrial Production of National Academy of Agrarian Sciences of Ukraine, Poltava, Ukraine*

*** *Kherson State Agricultural University, Kherson, Ukraine*

**** *Institute of Agriculture of the Carpathian region of National Academy of Agrarian Sciences of Ukraine, Obroshino, Ukraine*

Article info

Received 04.07.2026

Received in revised form 23.08.2026

Accepted 12.09.2026

Poltava State Agrarian University,
Skovorody st., 1/3, Poltava, 36003,
Ukraine. Tel.: +38-096-944-98-12.
E-mail:
pavlo.vashchenko@pdau.edu.ua

Institute of Pig Breeding and
Agroindustrial Production of
National Academy of Agrarian
Sciences of Ukraine, Shvedska
Mohyla st., 1, Poltava, 36000,
Ukraine. Tel.: +38-066-727-24-93.
E-mail: pigbreeding@ukr.net

Kherson State Agricultural
University, Sretenskaya st, 23,
Kherson, 73006, Ukraine.
Tel.: +38-066-586-60-25.
E-mail: i.o.balabanova@ukr.net

Institute of Agriculture of the
Carpathian region of National
Academy of Agrarian Sciences
of Ukraine, Mykhailo Hrushevsky
st., 5, Obroshino, Lviv Region,
81115, Ukraine.
Tel.: +38-067-747-18-36.
E-mail: stadnytskaolha@ukr.net

*Vashchenko, P. A., Polishchuk, V. A., Tsereniuk, O. M., Balabanova, I. A., Stadnytska, O. I., Shaferivskyi, B. S., Fesenko, O. G., & Polishchuk, A. A. (2026). Polymorphism of the *ADRB3* gene in Ukrainian Red-and-White Dairy cattle and its association with economically important traits. Regulatory Mechanisms in Biosystems, 17(9), e26165. doi:10.15421/0226165*

Marker-assisted selection (MAS) is a promising approach for identifying genetic variants associated with economically important traits in livestock. The β 3-adrenergic receptor gene (*ADRB3*) is involved in the regulation of energy metabolism and may influence growth, body development, and productive performance. This study aimed to characterize *ADRB3* polymorphism and evaluate its associations with milk productivity, live weight, and body conformation traits in Ukrainian Red-and-White Dairy cows. Genotyping was performed in 98 cows and their paternal sires. Six alleles (A–F) were identified, with allele A predominating (0.500), while alleles B and E were rare (0.015 each). The effective number of alleles was 3.019, the Shannon information index was 1.319, and the polymorphism information content (PIC) was 0.624, indicating high genetic variability. All 96 genotyped cows were heterozygous, with observed heterozygosity of 1.000 and a negative fixation index (-0.495). All six paternal sires had the AA genotype, indicating that the alternative alleles in daughters were inherited from the maternal side. Association analysis included 90 cows with AC, AD, and AF genotypes; rare AB and AE groups were excluded because of their small size and deviation from normality. Significant genotype-associated differences were found for 305-day milk yield, live weight, sacral height, chest depth, chest width, chest girth, pelvic width, and cannon bone girth. Cows with the AC genotype had 662.2 kg (10.6%) higher 305-day milk yield and 7.47 kg (1.5%) greater live weight compared with AD cows. The AC genotype was also associated with greater sacral height and pelvic width, whereas AD cows showed greater chest dimensions, cannon bone girth, and body compactness. Significant differences were additionally detected for leg length, pelvic–thoracic, compactness, and bone indexes. The findings indicate that *ADRB3* polymorphism may be a potential marker of variation in milk productivity, live weight, and body conformation in Ukrainian Red-and-White Dairy cattle. However, the specific pedigree structure and limited representation of sire families require cautious interpretation. Further studies in larger and independent populations are needed to validate these associations and clarify their biological basis.

Keywords: marker-assisted selection; milk yield; live weight; body conformation; genetic variability; β 3-adrenergic receptor.

Introduction

Modern livestock breeding increasingly relies on molecular genetic information to complement conventional phenotypic selection and accelerate genetic improvement. Marker-assisted selection (MAS) enables the identification and preferential use of animals carrying genetic variants associated with desirable traits, thereby increasing the accuracy and efficiency of selection and potentially shortening the generation interval (Lamido et al., 2024; Sharma et al., 2024; Vashchenko et al., 2025). Its application is particularly valuable for traits that are difficult, costly, or time-consuming to measure, or that can only be assessed later in life. By facilitating more precise selection for productivity, health, reproduction, feed efficiency, and adaptation, molecular breeding approaches can contribute to sustained improvement in the biological and economic efficiency of livestock production. Moreover, genetic improvement in production efficiency has implications beyond animal productivity. Selection for greater efficiency of resource use can help produce more animal-derived food from a given amount of land and other resources, while the incorporation of environmental traits into breeding objectives creates opportunities to reduce greenhouse gas emissions per unit of animal product. In dairy cattle, genetic selection for reduced methane emissions is increasingly considered a long-term and cumulative strategy for mitigating the environmental footprint of production (Zos-Kior et al., 2016; Brockova et al., 2021; Zos-Kior et al., 2021).

Marker-assisted selection can be based on different types of molecular markers and approaches, including restriction fragment length polymorphisms (RFLPs), microsatellites, insertion–deletion polymorphisms (indels), and single nucleotide polymorphisms (SNPs), with markers differing in abundance, polymorphism, genotyping requirements, and their degree of linkage with loci affecting the trait of interest (Bhatia & Bajwa, 2022; Saienko et al., 2023; Mustafa et al., 2026). Marker-assisted selection has progressively evolved from the use of individual markers linked to specific loci toward genome-wide approaches (Matiuk et al., 2025). Genomic selection (GS) represents a major advancement in this development, extending the principles of MAS to the simultaneous analysis of thousands of markers distributed across the entire genome. By estimating the combined effects of genome-wide markers, GS enables the prediction of genomic breeding values without requiring prior identification of the specific quantitative trait loci underlying a complex phenotype (Goddard & Hayes, 2007; Meuwissen et al., 2016; Zhu et al., 2025).

The development of GS has been recognized as one of the major advances in modern breeding methodology, forming part of a broader group of transformative approaches that have reshaped both crop and livestock improvement, alongside association mapping, QTL mapping, high-throughput phenotyping, envirotyping, transgenesis, and genome editing (Bernardo, 2016; Xu et al., 2020; Sharma et al., 2024). In livestock, the implementation of GS has substantially expanded the use of genomic information in breeding programs, par-

ticularly for complex traits controlled by numerous loci with small individual effects.

However, targeted marker-based approaches remain highly relevant when investigating candidate genes or specific genomic variants associated with economically important traits. Among the available marker types, SNPs have become particularly important because of their high abundance throughout the genome, suitability for high-throughput and automated genotyping, and compatibility with dense SNP arrays developed for major livestock species. Single nucleotide polymorphisms can serve as linked markers for tracking quantitative trait loci or, when located within functional genomic regions, as candidate functional variants potentially contributing directly to phenotypic variation. Their widespread distribution and relatively straightforward genotyping make them well suited for association studies and for incorporating molecular information into livestock breeding programs (Lourenco et al., 2020; Vashchenko et al., 2022; Sahana et al., 2025).

The *ADRB3* gene, encoding the β 3-adrenergic receptor, has attracted considerable attention as a candidate gene involved in the regulation of energy metabolism and body composition. The β 3-adrenergic receptor is predominantly expressed in adipose tissue, where activation by catecholamines stimulates lipolysis and thermogenesis and thereby contributes to the regulation of energy balance (de Jong et al., 2017). In humans, genetic variation in *ADRB3*, particularly the Trp64Arg substitution, has been investigated in relation to obesity and metabolic disorders. The Arg64 variant has been associated in some ethnic populations with increased body weight, altered obesity-related parameters, and an earlier onset of non-insulin-dependent diabetes mellitus, although the strength and direction of these associations have varied among populations. Experimental studies in rodents have provided additional evidence for a role of *ADRB3* in body composition and energy metabolism. Reduced *ADRB3* expression has been observed in the adipose tissue of obese rats, whereas disruption of *ADRB3* in mice was associated with increased body fat and reduced lean body mass. These findings suggest that alterations in *ADRB3* signaling may influence the partitioning of energy between adipose and lean tissues (Wu et al., 2012; Mei et al., 2018; Zeng et al., 2025).

In livestock, associations between *ADRB3* polymorphism and economically important traits have been reported primarily in sheep (Malau-Aduli & Kashani, 2015; Ali et al., 2023). Different *ADRB3* alleles have been associated with variation in birth weight, growth rate, carcass composition, and cold survival, although the effects were dependent in some cases on sex, birth rank, or genetic background. For example, particular sire-derived alleles were associated with differences in birth weight and growth rate, while variation in carcass composition was observed between specific allele groups. These findings indicate that *ADRB3* polymorphism may contribute to variation in traits related to growth, body composition, and energy metabolism in livestock (Wu et al., 2012).

In cattle, variation in the *ADRB3* gene has been characterized in the promoter region, where six allelic variants containing four single nucleotide polymorphisms (SNPs) and three nucleotide deletions / insertions were identified in a study of 737 animals representing New Zealand and Chinese cattle populations (Hu et al., 2010). Allele A was by far the most frequent variant, whereas alleles C, D, E, and F occurred at low frequencies and showed breed- or population-specific distributions. Notably, alleles D and E were detected exclusively in the Chinese cattle examined, while allele C was found only in Charolais and allele F in Hereford and Murray Grey cattle. The identified promoter variation was suggested to have potential regulatory effects, although its functional significance was not established. The authors also proposed that these variants could be linked to functional variation elsewhere within the *ADRB3* gene, emphasizing the need for further investigation of their biological and phenotypic consequences. However, despite the identification and characterization of *ADRB3* polymorphism in cattle, its associations with milk productivity, body development, and other economically important traits in dairy cattle have received considerably less attention.

The Ukrainian Red-and-White Dairy breed represents an important component of the national dairy cattle population and is charac-

terized by a substantial population size, ranking third among dairy breeds in Ukraine. Consequently, the productive performance and genetic potential of this breed have considerable economic significance for the Ukrainian dairy sector. Its relatively large population and established breeding structure also make it a suitable model for investigating genetic markers potentially associated with economically important traits (Machulnyi, 2018; Liubynskyi, 2023; Danshyn et al., 2025).

Therefore, the objective of the present study was to investigate the polymorphism of the *ADRB3* gene and its associations with milk productivity, live weight, linear body measurements, and body conformation indexes in Ukrainian Red-and-White Dairy cattle, thereby providing new insights into the potential contribution of *ADRB3* variation to economically important traits and its possible application in marker-assisted selection.

Materials and methods

The study was conducted in accordance with the ethical principles governing the treatment and use of animals in scientific research in Ukraine. The experimental procedures complied with the provisions of the Order of the First National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine On the Protection of Animals from Cruelty No. 3447-IV of 21 February 2006, including subsequent amendments. The research protocol was approved by the Committee for the Maintenance and Use of Animals at Poltava State Agrarian University. Particular attention was paid to minimizing animal disturbance and stress throughout the study. The cows were maintained under a loose-housing system in an enclosure that allowed them to move freely within the available space and express their normal locomotor and behavioral activities, without individual tethering or prolonged restraint.

The study involved 96 cows of the Ukrainian Red-and-White Dairy breed kept at the private agricultural company Ukraina in Myrhorod District of Poltava Oblast, Ukraine. All procedures were designed and performed with due consideration of animal welfare and with the aim of minimizing stress and discomfort. Blood sampling for molecular genetic analysis was performed using procedures intended to minimize handling time and animal discomfort. The health and general physiological condition of the animals were monitored throughout the study. The principles of Replacement, Reduction, and Refinement (3Rs), established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1985), were incorporated into the study design. Replacement was addressed by restricting the experimental procedures to those necessary to achieve the genetic and phenotypic objectives of the study, without introducing additional experimental interventions. Reduction was achieved by using the existing herd population and obtaining both genetic and phenotypic data from the same animals, thereby avoiding the involvement of additional animals. Refinement was ensured through minimally invasive blood sampling, appropriate handling procedures, and loose housing that allowed unrestricted movement within the enclosure and avoided prolonged individual restraint. The general health and condition of the animals were monitored throughout the study.

Particular emphasis was placed on the assessment of body size and conformation, as these characteristics may reflect differences in growth, body development, and the distribution of tissues among genetically distinct groups. A set of linear body measurements was recorded for each cow, including height at withers (cm), sacral height (cm), oblique body length (cm), chest depth (cm), chest width (cm), chest girth behind the shoulder blades (cm), pelvic width at the level of the ischial tuberosities (cm), and cannon bone girth (cm). Live weight (kg) was also recorded as an additional indicator of body development.

Height at withers was measured vertically from the ground to the highest point of the withers, whereas sacral height was determined from the ground to the highest point of the sacral region. Oblique body length was measured from the anterior edge of the shoulder joint to the ischial tuberosity. Chest depth was determined as the verti-

cal distance from the highest point of the withers to the lowest point of the sternum, while chest width was measured as the distance between the lateral surfaces of the shoulder joints. Chest girth behind the shoulder blades was measured with a tape around the thorax, just behind the shoulder blades. Pelvic width was determined as the distance between the ischial tuberosities. Cannon bone girth was measured around the middle portion of the metacarpus.

The linear measurements were obtained using a measuring stick with a scale division of 1 mm (Nasco Education, Ajax, Canada) and a measuring tape (Kruuse, Langeskov, Denmark). Live weight was determined using electronic livestock scales with an accuracy of 0.5 kg (Dnipro, Dnipro, Ukraine), immediately after morning milking and before feeding.

To characterize the proportional development of the body and the exterior type of the cows, the following body conformation indexes were calculated from the recorded linear body measurements (all expressed as a percentage): the leg length index was calculated as the ratio of the difference between height at withers and chest depth to height at withers; the body length index was determined as the ratio of oblique body length to height at withers; the pelvic-thoracic index was calculated as the ratio of chest width to pelvic width at the level of the ischial tuberosities; the thoracic index was determined as the ratio of chest width to chest depth; the compactness index was calculated as the ratio of chest girth behind the shoulder blades to oblique body length; the height proportion index was determined as the ratio of sacral height to height at withers; and the bone index was calculated as the ratio of cannon bone girth to height at withers.

Individual milk production data were automatically recorded during milking using milk yield sensors integrated into the GEA milking parlour system (GEA, Germany). For each cow, total milk yield per lactation (kg) and milk yield standardized to 305 days of lactation (kg) were recorded and used for subsequent statistical analysis.

The study was performed according to a structured experimental design comprising several consecutive stages. A schematic representation of the experimental workflow is provided in Figure 1.

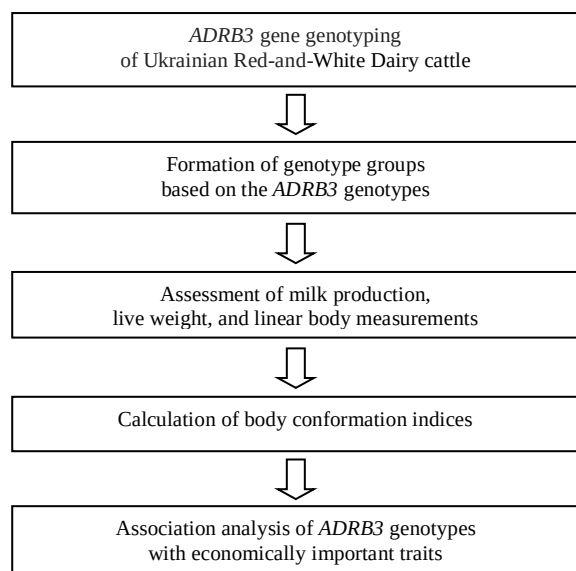


Fig. 1. Research organization scheme

Throughout the study, the cows received diets formulated in accordance with the feeding standards for dairy cattle described by Ibatullin et al. (2016). The diets were formulated taking into account an average live body weight of 550 kg and the changes in milk production occurring during the 305-day lactation. The average daily milk yield ranged from 26.5 kg during the second month of lactation to 15.1 kg during the tenth month. Since the applicable feeding standards provide nutrient requirements at 5 kg increments of daily milk production, the animals were supplied with three progressively adjusted dietary variants corresponding to milk yields of 15, 20, and 25 kg per day.

The nutritional profiles of the three diets differed according to the expected level of milk production. The diets supplied 156.6, 177.5, and 202.8 MJ of metabolizable energy; 2,759, 2,836, and 3,120 g of crude protein; 2,346, 2,269, and 2,340 g of rumen-degradable protein; 413, 567, and 780 g of rumen-undegradable protein; and 1,780, 1,830, and 2,047 g of digestible protein, respectively. The corresponding amounts of crude fiber were 4,272, 4,392, and 3,900 g, while starch and sugars were provided at levels of 2,403, 2,470, and 3,120 g and 1,602, 1,647, and 2,047 g, respectively. The diets also contained 570, 586, and 702 g of crude fat, together with 115.7, 119.0, and 126.8 g of calcium and 80.1, 82.3, and 87.8 g of phosphorus. Thus, the feeding program was adjusted to accommodate the changing nutritional requirements of cows throughout lactation rather than maintaining a uniform ration throughout the experimental period.

Seasonal changes in the composition of the feed ration were also taken into consideration. During the winter feeding period, the basal diet consisted of sainfoin, grass, and alfalfa hay, silage, fodder beet, and concentrate feeds. A protein–mineral–vitamin supplement incorporated at a rate of 4% was used to provide additional protein, mineral, and vitamin nutrients. During the summer period, the forage component was based on green maize harvested at the milk–wax stage, wilted alfalfa, Sudan grass, and oat hay, supplemented with barley straw and the same 4% protein–mineral–vitamin supplement. Throughout the experimental period, the cows had continuous access to fresh drinking water. The quantity of feed supplied was recorded daily for each experimental group, allowing the overall amount of feed provided to the animals to be monitored throughout the study. Because the animals were housed and fed as groups, individual feed intake was not measured.

The animals were maintained under loose-housing conditions in well-ventilated barns designed and operated in accordance with established zoohygienic requirements. This housing system allowed the cows to move freely within the available group area and avoided individual tethering, thereby reducing prolonged physical restraint during the production period. The combination of seasonally adapted forage sources and nutritionally balanced diets was maintained throughout the experimental period to provide consistent feeding conditions for the assessment of milk productivity and other phenotypic traits (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 blood samples using the Chelex 100 method (Bio-Rad Laboratories, Inc., USA), according to Walsh et al. (2013). The *ADRB3* gene fragment was amplified by polymerase chain reaction (PCR). The primer pair used for amplification was selected based on previously published information on polymorphism of the bovine *ADRB3* gene (Hu et al., 2010). The forward primer was 5'-GTCCTTTCCTTTACCTCCAG-3' and the reverse primer was 5'-GTCACACACAGCAGCTCCAC-3'. These primers amplified an approximately 900 bp fragment of the bovine *ADRB3* gene containing part of the promoter region and exon 1.

The PCR reaction was performed in a Tercyk-2 thermocycler (Tertsik TP4-PCR01, DNA-Technology, RF) using 0.5-mL Eppendorf microcentrifuge tubes (Eppendorf, Germany). The optimized PCR conditions included an annealing temperature of 58 $^{\circ}$ C, which enabled amplification of the expected 900 bp *ADRB3* fragment. The amplification program consisted of an initial denaturation at 94 $^{\circ}$ C for 3 min, followed by 35 cycles of denaturation at 94 $^{\circ}$ C for 30 s, primer annealing at 58 $^{\circ}$ C for 30 s, and elongation at 72 $^{\circ}$ C for 60 s. The reaction was completed by a final elongation step at 72 $^{\circ}$ C for 2 min.

Variability of the *ADRB3* amplicons was assessed using the single-strand conformation polymorphism (SSCP) technique, which allows the detection of sequence variation based on differences in the electrophoretic mobility of single-stranded DNA molecules caused by sequence-dependent conformational changes. The SSCP analysis was

performed following the methodological approach recommended for the detection of polymorphism in the bovine *ADRB3* gene by Hu et al. (2010).

For SSCP analysis, a 10 µL aliquot of the PCR amplicons was mixed with 3 µL of loading dye containing 30% glycerol and bromophenol blue and denatured at 95 °C for 5 min. The samples were then rapidly cooled on wet ice and loaded onto 8% acrylamide (37.5:1) gels. Electrophoresis was performed using a Cleaver electrophoresis system (Cleaver Scientific Ltd., England) at 275 V and a current of 76

mA for 90 min in 1× TBE (Tris-borate-EDTA) buffer. The gels were stained with ethidium bromide.

The SSCP profiles were used to identify sequence variants of the *ADRB3* gene according to the previously described patterns and allele characterization reported by Hu et al. (2010). In total, seven electrophoretic patterns were observed, of which five patterns (AB, AC, AE, AD, and AF) were identified based on the characteristic profiles associated with four single-nucleotide polymorphisms (SNPs) and two insertion/deletion variants, c.-429_430delAC and c.-468delC.



Fig. 2. Housing, feeding, and milking conditions of the experimental Ukrainian Red-and-White Dairy cows: *a* – cows in the outdoor loose-housing enclosure with access to feed; *b* – cows in the milking parlour (private agricultural company Ukraina, Poltava Oblast, Ukraine; Valentyn Polishchuk is shown during routine monitoring of the animals and the experimental conditions (photo by Pavlo Vashchenko, 2024)

Visualization of the electrophoretic profiles were visualized using a Clear View UV transilluminator (Cleaver Scientific Ltd., England) and documented photographically under ultraviolet illumination. An electropherogram of the 8% polyacrylamide gel showing the corresponding *ADRB3* patterns is presented in Figure 3.

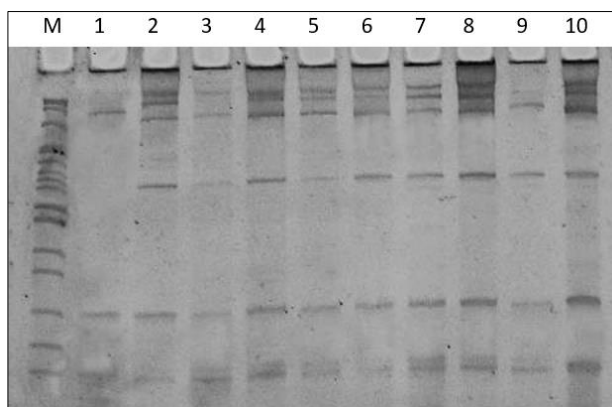


Fig. 3. Electropherogram of the *ADRB3* gene on an 8% polyacrylamide gel: 1 – AB; 2, 3, 5, 6, 7, 8, and 10 – AC; 4 – AF; 9 – AD; M – molecular weight marker, pBR322 DNA MspI Digest Ladder

Allele and genotype distributions were determined using the GenAlEx 6.0 software package (Peakall, Australia, 2012). The same software was used to estimate the polymorphism information content

(PIC) of the studied *ADRB3* locus and the fixation index (F) (Peakall, 2012). Observed genotype frequencies were compared with those expected under the corresponding theoretical distribution using the chi-square (χ^2) test. The fixation index was calculated according to the following formula: $F = (H_e - H_o) / H_e$, where H_e is the expected heterozygosity and H_o is the observed heterozygosity (Peakall, 2012).

Following the genotyping of the experimental cows, the genotypes of their paternal sires were additionally determined to clarify the inheritance pattern of the *ADRB3* alleles. Six sires were identified as fathers of the studied cows: Kandi (7 daughters), Kandi Red Tv Tl (22 daughters), Saphir/30 (1 daughter), Chaklun/5276 (9 daughters), Chop (15 daughters), and Chop/5836 (42 daughters). The sires were genotyped using the same PCR-SSCP procedure as that applied to the cows. Genomic DNA was extracted from frozen semen straws stored at the farm, and the resulting DNA was subsequently subjected to PCR amplification and SSCP analysis under the same conditions described above.

Statistical analysis of milk production, live weight, morphometric measurements, and body conformation indices was conducted with SAS/STAT® 15.1 (SAS Institute Inc., USA, 2018). Descriptive statistics are presented as arithmetic means with their standard errors ($\bar{x} \pm SE$). The effect of genotype on the investigated traits was assessed by one-way analysis of variance (ANOVA). Fisher's F-test was used to determine the statistical significance of variation among genotype groups relative to within-group variation. Where significant differences were detected, pairwise comparisons were performed using Tukey's honestly significant difference (HSD) test. Statistical significance was established at $P < 0.05$.

Results

Genetic analysis of the studied cattle population revealed a non-uniform distribution of alleles at the *ADRB3* locus. Allele A was predominant, whereas alleles B and E were detected at the lowest frequencies. The frequency of allele A was more than 33 times higher than that of alleles B and E, and approximately 2.6 times higher than that of alleles C and F. Among the identified genotypes, AC and AF were the most prevalent, together accounting for more than three-quarters of the animals examined. The remaining genotypes occurred considerably less frequently. The observed genotype distribution differed significantly from the expected distribution, as indicated by the highly significant χ^2 value (Table 1). The *ADRB3* locus exhibited a high level

of genetic variability in the studied population, with six alleles detected and the locus classified as polymorphic. The effective number of alleles was 3.019, indicating that the contribution of individual alleles to the observed genetic diversity was unequal. The Shannon information index reached 1.319, further confirming the substantial allelic diversity of the *ADRB3* locus.

Because all 96 genotyped cows were heterozygous at the *ADRB3* locus, the genotypes of their paternal sires were additionally analyzed to clarify the inheritance pattern of the observed alleles. All six sires identified as fathers of the studied cows were homozygous for allele A (genotype AA). Consequently, each daughter inherited an A allele from her sire, whereas the alternative allele observed in her heterozygous genotype must have been inherited from the maternal side.

Table 1

Allelic and genotypic composition of the *ADRB3* locus in Ukrainian Red-and-White Dairy cattle (n = 98)

Locus	Allele frequencies						Genotype frequencies					χ^2	P	Fixation Index
	A	B	C	D	E	F	AB	AC	AD	AE	AF			
<i>ADRB3</i>	0.500	0.015	0.194	0.087	0.015	0.189	0.031 (0.015)	0.388 (0.194)	0.173 (0.087)	0.031 (0.015)	0.378 (0.189)	98.0	3.12*10 ⁻¹⁴	-0.495

Note: values in parentheses represent the expected genotype distribution.

A pronounced excess of heterozygous individuals was therefore observed in the studied population. The observed heterozygosity was 1.000, whereas the expected heterozygosity and unbiased expected heterozygosity were 0.669 and 0.672, respectively. Thus, the observed level of heterozygosity substantially exceeded that expected based on the allele frequencies. This pattern was also reflected by the negative fixation index presented in Table 1, indicating a marked excess of heterozygous genotypes at the *ADRB3* locus. The polymorphism information content (PIC) of the *ADRB3* locus was 0.624, indicating a high level of polymorphism and marker informativeness. The allelic structure of the *ADRB3* locus in the studied population is presented in Figure 4.

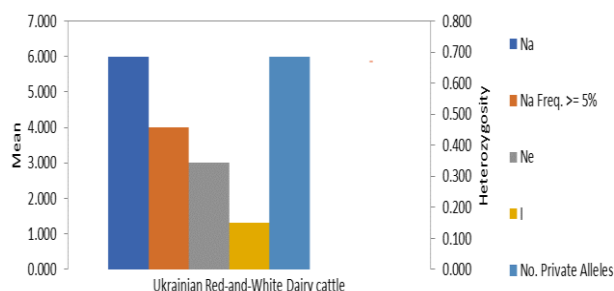


Fig. 4. Allelic patterns across populations

Six alleles (Na = 6) were identified, four of which occurred at frequencies of at least 5% (Na Freq. $\geq 5\%$ = 4). The effective number of alleles (Ne = 3.019) was considerably lower than the total number of observed alleles, reflecting unequal allele frequencies within the po-

pulation. The Shannon information index (I = 1.319) further indicates substantial allelic diversity. The analysis also identified six private alleles (No. Private Alleles = 6), whereas no locally common alleles occurring at frequencies of $\leq 5\%$ or $\leq 50\%$ were detected. The observed heterozygosity (Ho = 1.000) exceeded the expected heterozygosity (He = 0.669) and unbiased expected heterozygosity (uHe = 0.672), indicating a pronounced excess of heterozygous genotypes in the studied population.

Overall, the *ADRB3* locus demonstrated considerable allelic diversity and a high degree of polymorphism in the Ukrainian Red-and-White Dairy cattle population. The combination of a high PIC value, multiple alleles, and elevated heterozygosity indicates that this locus provides sufficient genetic variability for investigating potential associations between *ADRB3* polymorphism and economically important traits.

An association analysis was performed to evaluate the relationships between *ADRB3* genotypes and milk productivity, live weight, and selected linear body measurements in Ukrainian Red-and-White Dairy cows. Of the 98 animals genotyped at the *ADRB3* locus, 96 were included in the association analysis of productive and morphological traits. Two animals, one carrying the AC genotype and one carrying the AD genotype, were excluded from the association analysis because of limb injuries that occurred during the experimental period and could potentially affect the evaluated body conformation and productivity traits. Also, animal groups with AB and AE genotypes were not included in the association analysis due to the small number of animals with these genotypes and the deviation from a normal distribution. Thus, the association analysis was performed using 90 animals (Table 2).

Table 2

Effect of *ADRB3* genotype on milk productivity, live weight, and linear body measurements of Ukrainian Red-and-white cows (x $\pm$ SE)

Productive traits	Genotype					F	P
	AC (n = 37)	AD (n = 16)	AF (n = 37)	AB* (n = 3)	AE* (n = 3)		
Milk yield per lactation, kg	7684.9 $\pm$ 294.9	7495.4 $\pm$ 283.2	7486.4 $\pm$ 276.8	6930.7	8014.0	0.153	0.858
Milk yield for 305 days, kg	6885.7 $\pm$ 169.4 ^a	6223.50 $\pm$ 89.67 ^b	6464.49 $\pm$ 158.24 ^{ab}	6429.7	6644.0	3.531	3.35*10 ⁻²
Live weight, kg	497.16 $\pm$ 1.82 ^a	489.69 $\pm$ 1.85 ^b	491.59 $\pm$ 1.54 ^b	488.33	498.3	4.470	1.42*10 ⁻²
Height at withers, cm	139.81 $\pm$ 0.47	139.25 $\pm$ 0.71	139.22 $\pm$ 0.43	140.33	139.0	0.489	0.615
Sacral height, cm	145.49 $\pm$ 0.22 ^a	143.56 $\pm$ 0.41 ^b	144.16 $\pm$ 0.31 ^b	144.0	142.7	10.002	1.23*10 ⁻⁴
Oblique body length, cm	170.57 $\pm$ 0.88	169.00 $\pm$ 1.49	169.78 $\pm$ 1.48	174.33	169.7	0.288	0.751
Chest depth, cm	79.11 $\pm$ 0.58 ^a	82.06 $\pm$ 0.83 ^b	79.08 $\pm$ 0.67 ^a	82.3	79.3	4.135	1.93*10 ⁻²
Chest width, cm	53.81 $\pm$ 0.18 ^a	55.00 $\pm$ 0.26 ^b	53.92 $\pm$ 0.20 ^a	55.0	53.0	6.762	1.86*10 ⁻³
Chest girth behind the shoulder blades, cm	199.432 $\pm$ 0.532 ^a	203.875 $\pm$ 1.004 ^b	200.757 $\pm$ 0.432 ^b	204.000	199.667	11.053	5.28*10 ⁻⁵
Pelvic width at the level of the ischial tuberosities, cm	40.108 $\pm$ 0.218 ^a	39.125 $\pm$ 0.301 ^b	39.486 $\pm$ 0.200 ^{ab}	38.333	39.000	4.276	0.017
Cannon bone girth, cm	19.568 $\pm$ 0.099 ^a	19.813 $\pm$ 0.188 ^b	19.622 $\pm$ 0.081 ^a	19.667	20.000	3.901	0.02386

Note: different letters within each row indicate significant differences between groups according to Tukey's HSD test results; * – due to the small number of animals with this genotype and the deviation from a normal distribution, this group was excluded from the statistical analysis.

Significant differences among *ADRB3* genotype groups were observed for 305-day milk yield, live weight, sacral height, chest depth, chest width, chest girth behind the shoulder blades, pelvic width at the level of the ischial tuberosities, and cannon bone girth.

For 305-day milk yield, cows with the AC genotype had a significantly higher yield than cows with the AD genotype, with a difference of 662.2 kg (10.6%). The AF genotype showed an intermediate value and did not differ significantly from either AC or AD.

Significant differences were also observed in live weight. Cows with the AC genotype were 7.47 kg (1.5%) heavier than those with the AD genotype and 5.57 kg (1.1%) heavier than cows with the AF genotype. No significant difference was detected between the AD and AF groups.

Regarding body conformation, cows with the AC genotype had a greater sacral height than cows with the AD and AF genotypes, by 1.93 cm (1.3%) and 1.33 cm (0.9%), respectively. By contrast, cows with the AD genotype had 2.95 cm (3.7%) and 2.98 cm (3.8%) greater chest depth than both AC and AF cows, respectively. A similar pattern was observed for chest width, where AD cows exceeded AC and AF cows by 1.19 cm (2.2%) and 1.08 cm (2.0%), respectively.

Significant differences were also found for chest girth behind the shoulder blades. Cows with the AD genotype had a greater chest girth

than those with the AC and AF genotypes, by 4.44 cm (2.2%) and 3.12 cm (1.6%), respectively.

The pelvic width at the level of the ischial tuberosities was significantly greater in cows with the AC genotype, compared with cows with the AD genotype, by 0.98 cm (2.5%). The AF group had an intermediate value and did not differ significantly from either group.

Finally, cows with the AD genotype had a greater cannon bone girth than cows with the AC and AF genotypes, by 0.25 cm (1.3%) and 0.19 cm (1.0%), respectively.

Overall, the *ADRB3* genotype was associated with differences in several morphometric traits. Cows with the AC genotype were characterized by greater sacral height, pelvic width, live weight, and 305-day milk yield, whereas cows with the AD genotype had greater chest depth, chest width, chest girth, and cannon bone girth. No statistically significant differences were detected for total lactation milk yield, height at withers, or oblique body length among the analyzed genotype groups.

Individual linear measurements characterize the absolute size of the animals; however, the proportions between different body dimensions offer a more comprehensive assessment of conformation. Accordingly, a set of body conformation indexes was calculated to characterize the exterior type of the cows (Table 3).

Table 3

Variation in body conformation indexes among Ukrainian Red-and-White cows with different *ADRB3* genotypes ($\bar{x} \pm \text{SE}$)

Body composition index	Genotype					F	P
	AC (n = 37)	AD (n = 16)	AF (n = 37)	AB* (n = 3)	AE* (n = 3)		
Leg length, units	43.436 $\pm$ 1.702 ^a	41.082 $\pm$ 1.595 ^b	43.215 $\pm$ 2.247 ^a	41.357	42.974	9.010	2.78*10 ⁻⁴
Body length, units	121.98 $\pm$ 2.18	121.34 $\pm$ 2.38	121.94 $\pm$ 5.71	124.22	122.05	0.157	0.855
Pelvic–thoracic, units	134.30 $\pm$ 4.85 ^a	140.71 $\pm$ 5.31 ^b	136.65 $\pm$ 4.45 ^a	143.70	136.01	10.170	1.07*10 ⁻⁴
Thoracic, units	68.16 $\pm$ 3.37	67.11 $\pm$ 2.75	68.36 $\pm$ 3.79	66.99	66.98	0.750	0.476
Compactness, units	117.00 $\pm$ 3.09 ^a	120.74 $\pm$ 3.65 ^b	118.55 $\pm$ 6.34 ^{ab}	117.06	117.79	3.488	0.035
Height proportion, units	104.10 $\pm$ 2.34	103.14 $\pm$ 2.55	103.59 $\pm$ 2.48	102.65	102.74	0.959	0.387
Bone index, units	13.999 $\pm$ 0.442 ^a	14.408 $\pm$ 0.556 ^b	14.098 $\pm$ 0.415 ^{ab}	14.013	14.400	4.597	0.013

Note: different letters within each row indicate significant differences between groups according to Tukey's HSD test results; * – due to the small number of animals with this genotype and the deviation from a normal distribution, this group was excluded from the statistical analysis.

Statistically significant differences among the *ADRB3* genotype groups were identified for leg length, pelvic–thoracic, compactness, and bone indexes, whereas no significant differences were observed for body length, thoracic, or height proportion indexes.

For the leg length index, cows with the AC genotype had significantly higher values than those with the AD genotype, exceeding them by 2.35 units (5.73%). The AF genotype also showed a significantly higher leg length index than AD, with a difference of 2.13 units (5.19%), while no significant difference was detected between AC and AF cows.

A significant difference was also observed for the pelvic–thoracic index. Cows with the AD genotype had 6.41 (4.77%) and 4.06 units (2.97%) higher values than those with the AC and AF genotypes, respectively.

Regarding the compactness index, AD cows exceeded AC cows by 3.74 units (3.20%). The AF group had an intermediate value and did not differ significantly from either AC or AD. A similar pattern was observed for the bone index, for which cows with the AD genotype had significantly higher values than those with the AC genotype, by 0.41 units (2.92%), whereas the difference between AD and AF was not statistically significant.

Overall, the observed differences indicate that *ADRB3* polymorphism was associated primarily with variation in the relative proportions of the limbs, pelvic and thoracic regions, as well as body compactness and bone development. By contrast, body length, thoracic, and height proportion indexes remained relatively stable across the analyzed *ADRB3* genotypes.

Discussion

The present study revealed substantial genetic variation at the *ADRB3* locus in the Ukrainian Red-and-White Dairy cattle population, with six alleles identified and a high polymorphism information content. The allele distribution was markedly uneven, with allele A

predominating, while the remaining alleles occurred at considerably lower frequencies. The PIC value of 0.624 indicates a high level of polymorphism and marker informativeness, exceeding the threshold of 0.50 proposed by Botstein et al. (1980) for highly informative markers and supporting the suitability of the *ADRB3* locus for association analysis. Despite this predominance of allele A, all genotyped cows were heterozygous, resulting in an unusually high observed heterozygosity and a pronounced negative fixation index. This combination of high allelic diversity and complete heterozygosity is of particular interest and warrants consideration of the pedigree structure of the studied population.

The complete absence of homozygous individuals among the studied cows, despite the relatively high frequency of allele A, is particularly noteworthy. Genotyping of the six paternal sires demonstrated that all of them carried the *ADRB3* AA genotype. Consequently, the A allele was consistently transmitted from the paternal side, whereas the alternative alleles detected in the daughters must have originated from their dams. This finding provides a plausible explanation for the predominance of heterozygous genotypes in the studied group and indicates that the observed allele distribution cannot be interpreted solely from the genotype frequencies of the cows without considering their pedigree structure. At the same time, the present data do not allow the maternal origin of individual C, D, and F alleles to be assigned to particular ancestral breeds, since the dams were not genotyped. An additional consideration concerns the interpretation of the genotype–trait associations in relation to the pedigree structure of the studied population. Because all identified paternal sires were homozygous for the A allele, the alternative alleles in the heterozygous daughters were necessarily inherited from the maternal side. Consequently, the AC, AD, and AF genotypes largely represent different maternal allelic contributions within the studied group. Therefore, some of the observed phenotypic differences among genotypes may reflect maternal lineage or family effects rather than the direct functional effect of the *ADRB3* variants themselves. This possibility is particularly relevant

given the absence of homozygous individuals and the relatively limited number of paternal families represented in the sample. Further studies including genotyped dams, a larger number of sire families, and pedigree- or genomic-based models would be valuable for separating the effects of *ADRB3* genotype from family and population structure.

The genetic background of the Ukrainian Red-and-White Dairy breed may provide an important context for the allelic diversity observed at the *ADRB3* locus. The breed was developed through complex reproductive crossbreeding, with Simmental cattle constituting the maternal foundation and Holstein cattle providing the principal paternal component; in some breeding schemes, Ayrshire and Montbéliarde cattle were also incorporated (Kruhliak et al., 2015; Krugliak & Krugliak, 2023; Biriukova et al., 2025). The breed was officially approved in 1993, and its modern genealogical structure continues to involve genetic material from several breeds. Moreover, Holstein bulls have played a particularly prominent role in the reproduction of Ukrainian Red-and-White Dairy breeding stock, with earlier assessments reporting a substantial contribution of Holstein sires (Ruban, 1996; Gladiy et al., 2018; Pochukalin et al., 2024). In the present herd, all six identified paternal sires were Holstein bulls, whereas the studied cows represented the maternal component of this breeding population. The combination of a historically diverse breed foundation and continued use of Holstein sires may therefore contribute to the maintenance of multiple *ADRB3* alleles in the population. The marked differences in *ADRB3* variant frequencies among sheep breeds reported by Wu et al. (2012) also indicate that the allelic composition of this locus should be interpreted within the specific genetic background of each population rather than considered as a universal pattern across breeds. This consideration is particularly relevant to the present population, in which the observed *ADRB3* allele distribution may reflect the complex breed history and specific breeding structure of Ukrainian Red-and-White Dairy cattle. However, determining the specific ancestral origin of the C, D, and F alleles would require genotyping of dams and, preferably, a broader pedigree- and population-based analysis.

Despite the pronounced excess of heterozygous genotypes observed in the present population, the high frequency of the *ADRB3* A allele is consistent with previously reported patterns in cattle. Hu et al. (2010) found that allele A was the predominant variant across the cattle populations examined in their study, including populations from both New Zealand and China, and suggested that its widespread occurrence may reflect selective forces favoring the maintenance of this allele. Thus, the predominance of allele A observed in the Ukrainian Red-and-White Dairy cattle population does not appear to be an isolated feature of the studied herd but is broadly consistent with the distribution of *ADRB3* alleles reported in other cattle populations. At the same time, the complete absence of homozygous individuals in our sample represents a distinct feature of the present population and is more plausibly related to its particular pedigree structure and the use of AA sires than to the general frequency pattern of the *ADRB3* locus.

The associations observed between *ADRB3* genotypes and body conformation and productivity traits in the present study are broadly consistent with previous observations in sheep (Forrest et al., 2003; Yang et al., 2013; Ali et al., 2021), although the specific phenotypic effects cannot be considered directly equivalent across species. Cows carrying the AC genotype were characterized by higher 305-day milk yield, greater live weight, greater sacral height, and wider pelvic development, combined with relatively less pronounced chest dimensions, corresponding overall to a more pronounced dairy-type body conformation. The fact that a significant association was detected for 305-day milk yield but not for total lactation yield is also noteworthy. Standardization to a 305-day lactation period reduces the influence of differences in lactation duration and therefore provides a more comparable measure of productive performance among animals. The absence of a significant association with total lactation yield may consequently reflect the greater variability of lactation length within the studied population, which can mask genotype-related differences in standardized milk production. The association of the C allele with

greater body mass is of particular interest in light of the findings of Forrest et al. (2003), who reported that paternal inheritance of the C allele in sheep was associated with greater birth weight of single-born lambs compared with inheritance of the D allele. Similarly, Yang et al. (2013) demonstrated that ovine *ADRB3* haplotypes were associated with variation in weaning weight and postweaning growth, with the presence of the C-a haplotype being associated with increased postweaning growth, whereas other haplotypes were associated with reduced growth. Taken together, these studies suggest that *ADRB3* variation may contribute to inter-individual differences in body growth and development across livestock species. Further evidence for the involvement of *ADRB3* variation in growth-related traits was provided by Ali et al. (2021) in Kajli sheep. In that study, four synonymous SNPs identified within the ovine *ADRB3* gene were investigated, and two variants (c.130C>T and c.294C>G) showed significant associations with 180-day body weight. Notably, the *ADRB3* polymorphisms investigated by Ali et al. (2021) were not in Hardy-Weinberg equilibrium, indicating a non-random distribution of genotypes in the studied sheep population. Although the specific variants and phenotypic traits differed from those examined in the present study, the association of *ADRB3* polymorphism with body weight provides additional evidence that genetic variation in this locus may be related to economically important growth and production characteristics in livestock. Together with the findings reported in sheep by Forrest et al. (2003) and Yang et al. (2013), these results support the potential relevance of *ADRB3* as a candidate gene for variation in body development and production-related traits, while emphasizing the need for species- and population-specific validation of individual genotype-phenotype associations.

In the present study, however, the effects associated with the different genotypes were not limited to live weight. The AC genotype was associated with a combination of higher milk productivity, greater sacral height, wider pelvic development, and relatively smaller chest dimensions, indicating a more pronounced dairy-type conformation. By contrast, AD cows showed greater chest depth, chest width, chest girth, cannon bone girth, and compactness, accompanied by lower live weight and 305-day milk yield. The pattern of associations across individual measurements and conformation indexes suggests that the observed genotype differences were related not simply to overall body size, but to differences in body proportions and structural type. The AC genotype was characterized by a relatively taller and more dairy-oriented conformation, reflected by greater sacral height and pelvic development together with less pronounced chest dimensions and compactness. By contrast, the AD genotype was associated with a deeper, wider, more compact and more strongly boned body. Thus, the contrasting AC and AD phenotypes appear to represent differences in body architecture rather than a uniform increase or decrease in overall body size. This contrasting pattern may indicate that *ADRB3* variation is associated not with a uniform change in body size, but with differences in the relative development of body regions and, potentially, in the allocation of metabolic resources among growth, body tissue development, and milk production. Importantly, the absence of significant differences for several measurements and conformation indexes indicates that the observed associations were trait-specific rather than reflecting a generalized effect of *ADRB3* genotype on body size. In particular, the lack of differences in withers height, oblique body length, body length index, thoracic index, and height proportion suggests that the genetic associations were more closely related to selected aspects of body structure and proportional development. The findings of Yang et al. (2013), who observed both positive and negative associations of different *ADRB3* haplotypes with growth, provide indirect support for such genotype-dependent effects. Nevertheless, because the ovine studies investigated different *ADRB3* variants and growth or carcass-related traits, further studies combining genotyping, detailed production phenotypes, and indicators of body composition are required to determine whether the associations observed in cattle reflect functional effects of the identified variants or linkage with causative mutations.

The interpretation of genotype-specific associations is also constrained by the low frequency of several genotypes. In particular, the

AB and AE groups each comprised only three animals and were therefore excluded from the formal association analysis because of insufficient sample size and deviation from normality. Consequently, the present statistical comparisons primarily concern the AC, AD, and AF genotypes, and the phenotypic effects of the rare B- and E-associated variants cannot be reliably evaluated. Increasing the sample size and including additional populations would be necessary to assess the effects of these rare variants.

Taken together, the available evidence indicates that *ADRB3* variation is characterized by substantial population-specific diversity and may be associated with multiple aspects of economically important phenotypic variation, particularly growth, body development, and production-related traits. The present study extends these observations to Ukrainian Red-and-White Dairy cattle by demonstrating genotype-associated differences in standardized milk yield, live weight, and several structural and conformation traits.

Overall, the present findings support the relevance of the *ADRB3* locus as a candidate genetic marker for variation in economically important traits in Ukrainian Red-and-White Dairy cattle. The observed associations were most evident for 305-day milk yield, live weight, and traits describing the relative development of the pelvic, thoracic, and skeletal regions, suggesting that *ADRB3* variation may be related to both productive performance and body conformation. At the same time, the unusual pedigree structure of the studied population, the predominance of heterozygous genotypes, and the limited representation of rare genotypes require caution in interpreting these relationships as direct genetic effects. Determining the reproducibility and biological basis of the observed associations requires further validation in larger and independent cattle populations, including a greater number of sire and dam families and, where possible, genomic-based analyses.

Conclusions

The Ukrainian Red-and-White Dairy cattle population demonstrated substantial genetic variability at the *ADRB3* locus, with six alleles identified and a high polymorphism information content (PIC = 0.624). Allele A predominated (0.500), whereas alleles B and E were rare (0.015 each). All analyzed cows were heterozygous, resulting in exceptionally high observed heterozygosity ($H_o = 1.000$) and a negative fixation index ($F = -0.495$), which was associated with the specific pedigree structure of the studied population and the use of AA paternal sires.

The *ADRB3* genotype was significantly associated with 305-day milk yield, live weight, sacral height, chest depth, chest width, chest girth, pelvic width, and cannon bone girth ($P < 0.05$). Cows with the AC genotype had higher 305-day milk yield and live weight, greater sacral height, and wider pelvic development, whereas cows with the AD genotype showed greater chest dimensions, cannon bone girth, and body compactness. Significant differences were also observed for leg length, pelvic-thoracic, compactness, and bone indexes, indicating genotype-associated variation in body proportions and conformation.

The results indicate that *ADRB3* polymorphism may serve as a potential molecular marker for variation in milk productivity, live weight, and body conformation in Ukrainian Red-and-White Dairy cattle. However, the observed associations should be interpreted with caution because of the specific pedigree structure, the absence of homozygous cows, the limited number of sire families, and the low frequency of AB and AE genotypes. Further studies involving larger and independent populations, genotyped dams and a broader range of sire families, as well as pedigree- or genomic-based analyses, are required to validate the observed associations and clarify the biological contribution of *ADRB3* polymorphism to economically important traits.

The authors declare no conflicts of interest.

References

Ali, A., Javed, K., Zahoor, I., Anjum, K. M., & Sharif, N. (2023). Identification of polymorphisms in the *MSTN* and *ADRB3* genes associated with

- growth and ultrasound carcass traits in Kajli sheep. *Animal Biotechnology*, 34(4), 847–862.
- Bernardo, R. (1996). Best linear unbiased prediction of maize single-cross performance. *Crop Science*, 36(1), 50–56.
- Bhatia, D., & Bajwa, G. S. (2022). Molecular marker techniques and recent advancements. In: Sonah, H., Goyal, V., Shivaraj, S. M., & Deshmukh, R. K. (Eds.). *Genotyping by sequencing for crop improvement*. John Wiley & Sons Ltd., Hoboken. Pp. 1–21.
- Biriukova, O. D., Polupan, Y. P., Krugliak, A. P., Kovalenko, G. S., Melnik, Y. F., Krugliak, T. O., & Pryima, S. V. (2025). Genesis, status and prospects of dairy cattle breeding in Ukraine. *Animal Breeding and Genetics*, 70, 18–49.
- 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., & Rubezhanska, 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.
- Danshyn, V., Ruban, S., & Nazarenko, V. (2025). Modern approaches and prospects for the genetic evaluation of dairy cattle in breeding programmes. *Scientific Reports of the National University of Life and Environmental Sciences of Ukraine*, 21(3), 9–23.
- de Jong, J. M., Wouters, R. T., Boulet, N., Cannon, B., Nedergaard, J., & Petrovic, N. (2017). The β 3-adrenergic receptor is dispensable for browning of adipose tissues. *American Journal of Physiology – Endocrinology and Metabolism*, 312(6), E508–E518.
- Forrest, R. H., Hickford, J. G. H., Hogan, A., & Frampton, C. (2003). Polymorphism at the ovine β 3-adrenergic receptor locus: Associations with birth weight, growth rate, carcass composition and cold survival. *Animal Genetics*, 34, 19–25.
- Gladys, M. V., Polupan, Y. P., Bazyshina, I. V., Pochukalyn, A. E., Koval, T. P., Bezruchenko, I. M., Polupan, N. L., & Mikhaylenko, N. G. (2018). Genesis and prospects of red dairy cattle in Ukraine. *Animal Breeding and Genetics*, 51, 41–60.
- Goddard, M. E., & Hayes, B. (2007). Genomic selection. *Journal of Animal Breeding and Genetics*, 124(6), 323–330.
- Hu, J., Zhou, H., Smyth, A., Luo, Y., & Hickford, J. G. (2010). Polymorphism of the bovine *ADRB3* gene. *Molecular Biology Reports*, 37(7), 3389–3392.
- 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., Zhukulenko, I. S., Kostenko, V. I., Boiarчук, S. V. (2016). *Dovidnyk z povnotsinnoyi hodivli sil'skohospodarskykh tvaryn* [Handbook of balanced feeding of farm animals]. Ahrama Nauka, Kyiv (in Ukrainian).
- Krugliak, T. O., & Krugliak, A. P. (2023). Milk productivity of cows of the Ukrainian Red-and-White Dairy breed depends on the origin and compatibility of the bulls. *Animal Breeding and Genetics*, 66, 70–78.
- Kruhliak, A. P., Biriukova, O. D., Kovalenko, H. S., & Kruhliak, T. O. (2015). *Ukrainska chervono-riaba molochna poroda – rezultat realizatsiyi novoyi teoriiy 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).
- Lamido, M., Alade, N. K., Kembe, N. D., & Mukaddas, J. (2024). Marker assisted selection in livestock breeding: A review. *Nigerian Journal of Animal Production*, 2024, 113–116.
- Liubynskyi, O. I. (2023). Characteristics of the growth of heifers of different lines of the Bukoviny factory type of the Ukrainian Red-And-White Dairy breed. *Animal Breeding and Genetics*, 66, 86–94.
- Lourenco, D., Legarra, A., Tsuruta, S., Masuda, Y., Aguilar, I., & Misztal, I. (2020). Single-step genomic evaluations from theory to practice: Using SNP chips and sequence data in *BLUPF90*. *Genes*, 11(7), 790.
- 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.
- Malau-Aduli, A. E. O., & Kashani, A. (2015). Molecular genetics-nutrition interactions in the expression of *AANAT*, *ADRB3*, *BTG2* and *FASN* genes in the heart, kidney and liver of Australian lambs supplemented with *Spirulina* (*Arthrospira platensis*). *Genes and Genomics*, 37(7), 633–644.
- Matiuk, V. V., Saienko, A. M., Vashchenko, P. A., Slyenko, V. H., Fesenko, O. G., Peka, M. Y., & Tsereniuk, O. M. (2025). Association of polymorphisms in estrogen and prolactin receptor genes with reproductive traits in sows of rare breeds. *Regulatory Mechanisms in Biosystems*, 33(1), e25033.
- Mei, C. G., Gui, L. S., Wang, H. C., Tian, W. Q., Li, Y. K., & Zan, L. S. (2018). Polymorphisms in adrenergic receptor genes in Qinchuan cattle show associations with selected carcass traits. *Meat Science*, 135, 166–173.

- Meuwissen, T., Hayes, B., & Goddard, M. (2016). Genomic selection: A paradigm shift in animal breeding. *Animal Frontiers*, 6(1), 6–14.
- Mustafa, R. J., Khaleel, A. W., & Ezzulddin, T. A. (2026). Molecular technologies in animal improvement. *Istanus Journal on Applied Biological Sciences*, 2(1), e3.
- 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.
- Pochukalin, A. Y., Pryima, S. V., & Romanova, O. V. (2024). Breeding achievements of Ukraine (past, present): Breeds, types and lines of agricultural animals. *Animal Breeding and Genetics*, 67, 140–163.
- Ruban, J. D. (1996). Conservation of genetic stock of cattle breeds in the Ukraine. *Animal Genetic Resources*, 18, 79–91.
- Sahana, V. N., Gowane, G. R., Nayee, N., Khan, K. D., Yadav, A., Paul, R., Vohra, V., & Alex, R. (2025). Shared SNP effects across breeds increase the genomic prediction accuracy for numerically small breeds. *Scientific Reports*, 15, 31421.
- Saienko, A., Peka, M., Tsereniuk, O., Babicz, M., Kropiwiec-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.
- Sharma, P., Doultani, 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.
- Vashchenko, P. A., Skyba, M. M., Saienko, A. M., Slynko, V. H., Shaferivskyi, B. S., Myronenko, O. I., Stadnytska, O. I., & Tsereniuk, O. M. (2025). Association of *PIT1* gene polymorphism with milk productivity and body conformation traits in dairy cattle. *Regulatory Mechanisms in Biosystems*, 16(4), e25195.
- 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.
- 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.
- Wu, J., Qiao, L., Liu, J., Yuan, Y., & Liu, W. (2012). SNP variation in *ADRB3* gene reflects the breed difference of sheep populations. *Molecular Biology Reports*, 39(8), 8395–8403.
- Xu, Y., Liu, X., Fu, J., Wang, H., Wang, J., Huang, C., Prasanna, B. M., Olsen, M. S., Wang, G., & Zhang, A. (2020). Enhancing genetic gain through genomic selection: From livestock to plants. *Plant Communications*, 1(1), 100005.
- Yang, G., Zhou, H., Forrest, R. H., Fang, Q., Luo, Y., & Hickford, J. G. (2013). Haplotypes of the ovine *ADRB3* gene (*ADRB3*) and their association with post-weaning growth in New Zealand Suffolk sheep. *Molecular Biology Reports*, 40(8), 4805–4810.
- Zeng, Y., Song, X., Chen, Q., Gu, Y., Zhang, J., Zhou, T., Li, Z., Wang, T., Chang, L., Yao, H., Wang, Y., Miao, L., Qian, L., Guo, T., Zhang, Y., Rodriguez-Fernandez, S., Vidal-Puig, A., & Xu, Y. (2025). The suprachiasmatic nucleus regulates brown fat thermogenesis in male mice through an adrenergic receptor *ADRB3-S100B* signaling pathway. *PLoS Biology*, 23(12), e3003534.
- Zhu, L., Yang, X., Xian, Y., Jiang, W., Pu, X., Wang, S., Cheng, J., Niu, L., Zhao, Y., Chen, L., Zhou, X., Wang, Y., Gan, M., Zhu, L., & Shen, L. (2025). Empowering precision livestock farming: Artificial intelligence applications in animal genomic breeding and multi-dimensional phenotypic measurement. *Smart Agricultural Technology*, 2025, 101655.
- 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., Hnatenko, I., 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.