INTERIM SCIENTIFIC REPORT

on the implementation of the project 110PHE/2025

"TransformDairyNet: Working together to upscale Cow-Calf-Contact dairy production and beyond"code PN-IV-P8-8.1-PRE-HE-

ORG-2025-0265

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Contractor:	Research and Development Institute for Bovine Balotesti (ICDCB)				
Phase 1:	Strengthening the institutional research capacity of ICDCB Balotești				
Implementation period:	14.07.2025-31.12.2025				
Acronym:	TransformDairyNet				
Project code:	PN-IV-P8-8.1-PRE-HE-ORG-2025-0265				

110PHE/2025

Contract number:

<u>AIM OF THE 110PHE / 2025 PROJECT</u> is represented by the strengthening of ICDCB Balotești's institutional capacity for the internationalization of research activities in the field of bovine welfare.

ACTIVITIES and OBJECTIVES of the *TransformDairyNet* **PROJECT are:**

- i) use of precision livestock farming (PLF) tools in monitoring cattle health (e.g., infrared thermography, accelerometry data);
- ii) advancing bioacoustics research in cattle by analyzing key vocal parameters across different physiological categories to assess welfare levels;
- iii) application of artificial intelligence (machine learning) processes in the study of cattle and buffalo behaviour;
- iv) analysis of stress biomarkers in cattle and their correlation with rearing and production systems (e.g., cow-calf contact studies);
- v) professional development of the institute's human resources through participation in international conferences and research internships, with active involvement of young researchers;
- vi) organization of a scientific event with international participation to support scientific networking and the development of future collaborations for European project proposals;
- vii) publication of scientific articles in ISI Web of Science—indexed journals and increased international visibility of research outcomes (open access).

ACTIVITIES implemented in Phase 1:

Activity 1.1 - Attending international conferences;

Activity 1.2 - Publishing scientific articles in Web of Science journals.

RESULTS OBTAINED IN PHASE 1 OF IMPLEMENTATION: PARTICIPATION IN 4 INTERNATIONAL CONFERENCES WITH PRESENTATION OF RESULTS (I, II, III, IV); PUBLICATION OF 3 ARTICLES IN WEB OF SCIENCE JOURNALS (V, VI, VII); SUBMISSION OF A ROMANIA–FRANCE BILATERAL PROJECT PROPOSAL (2025 CALL).

RESULTS PLANNED FOR PHASE 1 (according to the project implementation plan):

- Dissemination of project outcomes through attendance at international conferences;
- Publishing scientific articles in Web of Science journals.

The implementation of the TransformDairyNet project activities foreseen under Stage 1 took place predominantly within the Experimental Farm and the Production Systems Laboratory of the Research and Development Institute for Bovine Balotesti, and to a lesser extent within the Experimental Farm of the Research and Development Station for Bovine Arad (exclusively for the genome-wide association study on milking reactivity in Romanian Spotted cows).

In order to comply with current legislation and international best practices for research involving animals, ethical approval was obtained from the Ethics Committee established at the level of ICDCB Balotești for the monitoring of project 110PHE / 2025. Furthermore, all project activities complied with Directive 2010/63/EU on the protection of animals used for scientific purposes.

I. <u>AI APPROACHES FOR DETECTION AND REMOVAL OF ENVIRONMENTAL</u>

SOUNDS IN AUDIO RECORDINGS TO ENHANCE AUTOMATED ANNOTATION AND

EXTRACTION OF CATTLE VOCAL PARAMETERS (Proceedings of the 58th Congress of the International Society for Applied Ethology (ISAE), p. 400, 4-8.08.2025, Utrecht, Olanda, authors: Mincu-Iorga M., Gavojdian D., Nicolae I., Zamansky A., Lazebnik T.)

Machine learning (ML) techniques are increasingly being used in cattle vocalization research, addressing tasks such as distinguishing between high and low frequency calls, identifying ingestive behaviour sounds or classifying calls like oestrus and coughs; however, although some studies addressed cattle sound classification from background noise, extraction of cow sounds from complex on-farm recordings remains limited, highlighting the need for annotated vocalization datasets of interest. The main objective of this study was to develop a model for cattle vocalization extraction, while filtering out unwanted environmental sounds (e.g., farming equipment, birds, rodents, metal clanging, wind) from livestock audio recordings, using ML techniques. Our dataset contained both cattle sounds and interfering environmental noises, that have been manually annotated in previous audio-recordings (over 120 hours). The study was conducted at the experimental farm of the Research and Development Institute for Bovine in Balotesti, Romania. The raw audio recordings were collected from 20 multiparous dairy cows, each individually recorded for 4 hours post-milking while tethered in identical barns. We manually cleaned, preprocessed and annotated 71 cow calls and 64 environmental sounds. In the first step we developed a deep learning (DL) classifier for the binary classification of cow/environmental sounds. We then used this classifier with a sliding window technique for the detection of the cow sounds in raw audio files. For the first classification task we adopted the Wavegram-Logmel-CNN modelas a pre-trained audio model, fine-tunning it using our data, reaching 0.9231 accuracy. For the second detection task, we used a window in the size of half of the duration of an average cow sound, reaching 0.77 accuracy. These promising results can be further improved using more sophisticated preprocessing techniques. The outcomes of this study have practical applications in welfare research, enhancing extraction of vocalizations in bioacoustics studies. Using it significantly reduces the time required for annotating vocalization datasets, enhancing efficiency in sound data processing. Moreover, by enabling the automatic detection of vocal indicators linked to emotional states, it supports the development of behaviour-based tools for welfare monitoring.



Figure 1. Schematic representation of the experimental design

Table. 1 Performance Metrics

Measure	Value
Sensitivity	0.9286
Specificity	0.9167
Precision	0.9286
Negative Predictive Value	0.9167
False Positive Rate	0.0833
False Discovery Rate	0.0714
False Negative Rate	0.0714
Accuracy	0.9231
F1 Score	0.9286
Matthews Correlation Coefficient	0.8452

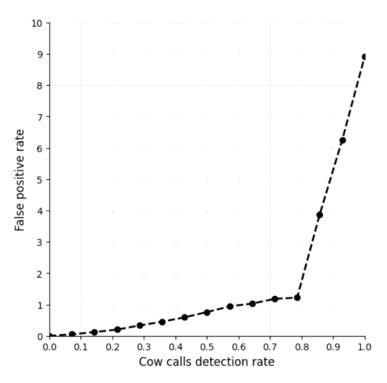


Figure 2. Cow calls detection rate

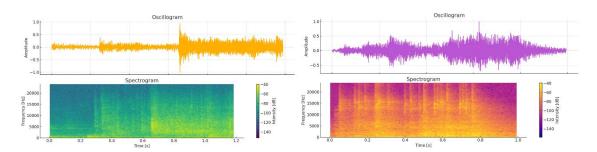


Figure 3. Original Environmental Sounds (Non-Cow Examples) Left: Defecation and Urination Sound & Right: Metal Clanging Sound

II. <u>GENOME-WIDE ASSOCIATION STUDIES FOR MILKING REACTIVITY IN</u>

<u>ROMANIAN SPOTTED CATTLE</u> (Book of Abstracts of the 76th European Federation of Animal Science, ISBN: 979-12-210-6769-9 vol. 39, p. 777, 25-29.08.2025, Innsbruck, Austria, authors: Minculorga M., Mizeranschi A.E., Gavojdian D., Nicolae I., Ilie D.E.)

Cattle behaviour during milking is a key trait affecting animal welfare and farm efficiency. Highly reactive cows exhibit excessive movement or resistance during milking, impacting milk yield and overall herd management.

Behavioural traits in cattle were shown to have a genetic component, however, their underlying mechanisms remain poorly understood.

This study aimed to identify genetic markers associated with milking reactivity in Romanian Spotted dual-purpose cattle breed using a genome-wide association study (GWAS). The experiment was conducted on a number of 185 cows, between 1st and 3rd parities, milked twice per day in a 'herring-bone' 2x14 parlour. Milking reactivity was scored on a 5-point scale, where lower values indicated calmness and higher values indicated nervousness. The Axiom Bovine v3 SNP-chip was used to genotype 88 SNP markers belonging to 24 candidate genes, which were previously found to be associated with behavioural traits.

Principal component analysis (PCA) was performed to assess population structure and GWAS was conducted using a mixed linear model, adjusting for parity and the first 10 principal components. Five significant SNPs were associated with milking reactivity, located within the USH2A, ADAMTS7, TBC1D2B and ZMAT4 genes, previously identified as candidate genes associated with milking temperament.

These findings highlight the polygenic nature of milking reactivity, with genes involved in sensory perception, motor control and intracellular signalling influencing this trait. Understanding these genetic factors could support breeding strategies aimed at improving milkability and stress resilience in dual-purpose cattle. Future studies should incorporate whole-genome sequencing to refine genetic associations and validate these results in larger populations.

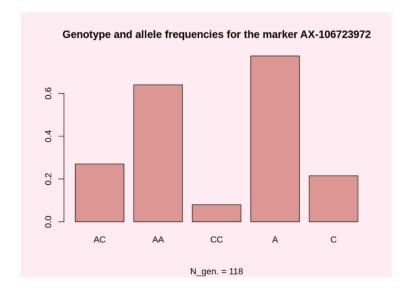


Figure 4. Genotype and Allele Frequency Distribution for Marker AX-106723972

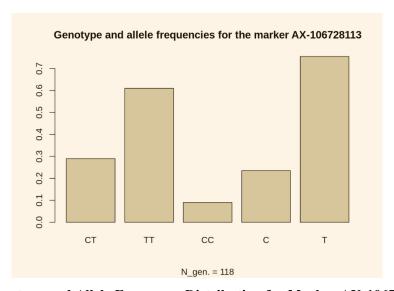


Figure 5. Genotype and Allele Frequency Distribution for Marker AX-106728113

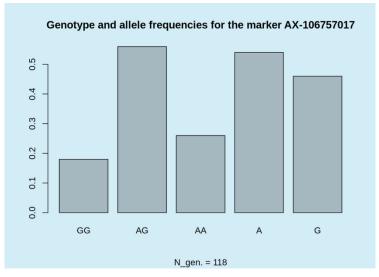


Figure 6. Genotype and Allele Frequency Distribution for Marker AX-106757017

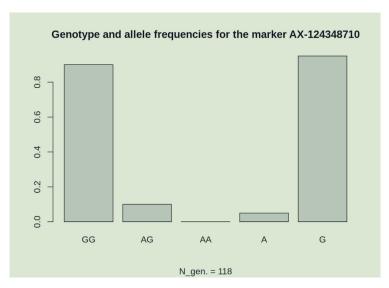


Figure 7. Genotype and Allele Frequency Distribution for Marker AX-124348710

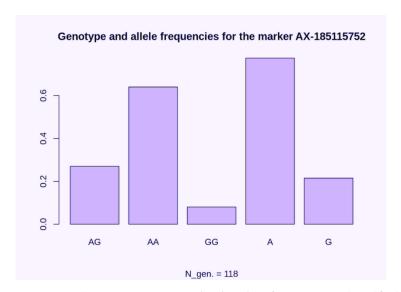


Figure 8. Genotype and Allele Frequency Distribution for Marker AX-185115752

III. MACHINE LEARNING-BASED ANALYSIS OF DAIRY COW VOCAL BEHAVIOUR (Book of abstract of the International Scientific Symposium "Young People and Multidisciplinary Research in Applied Life Sciences" ISSN: 2821-4285, ISSN - L: 2821-4285 p. 47, 13-14.11.2025, Timisoara, authors: Mincu-Iorga M., Gavojdian D.)

Understanding animal vocalizations through multi-source data fusion is pivotal for assessing emotional states and advancing animal welfare in precision livestock farming. This study explores dairy cattle contact calls using integrated multi-modal approaches, combining acoustic pattern extraction, sequential analysis and acoustic feature profiling. The Natural Language Processing-based WHISPER model was employed to convert audio recordings of cow vocalizations into acoustic-to-text sequences.

By integrating acoustic characteristics such as frequency, duration, and intensity with these sequential acoustic patterns, a robust representation of cow vocalizations was developed. Leveraging data fusion techniques within a specifically developed ontology, we categorized vocalizations into high-frequency calls (HFC) putatively associated with arousal or agitation, and low-frequency calls (LFC) indicative of calm or content states. Multi-dimensional data analysis revealed specific acoustic indicators of the two call types, including distinct frequency patterns and spectral attributes. Evaluating acoustic and sequential patterns from vocalizations of 20 lactating cows facilitated differentiation in calling behaviors. Advanced machine learning methods, including Random Forest, Support Vector Machine (SVM), and Recurrent Neural Networks (RNN), effectively analyzed and fused multi-source acoustic data, resulting in classification accuracies of 97.25% (Random Forest), 98.35% (SVM), and 88.00% (RNN). The F1-scores for identifying arousal were 0.98 (Random Forest) and 0.99 (SVM). To our knowledge, this is the first study that combines acoustic and symbolic data to analyze vocalizations of dairy cows in order to develop a non-invasive classification model using multi-modal machine learning.

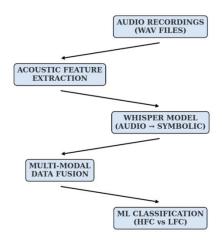


Figure 9. Methodological Workflow for Dairy Cattle Vocalization Classification

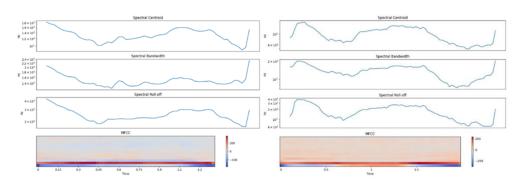


Figure 10. Left side – Spectral analysis of a typical Low-Frequency Call (LFC); Right side – Spectral analysis of a typical High-Frequency Call (HFC)

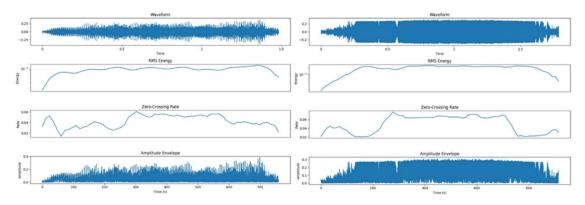


Figure 11. Left side – Amplitude and energy profile of a Low-Frequency Call (LFC), showing lower RMS energy and smoother transitions supporting a calm vocal classification; Right side – Amplitude and energy profile of a High-Frequency Call (HFC), with higher RMS energy and variability reflecting vocal strain and emotional intensity

IV. EFFECTS OF NIGELLA SATIVA OIL SUPPLEMENTATION ON METHANE EMISSIONS

<u>IN DAIRY CATTLE</u> (13th International Conference, Agriculture & Food 2025, International Scientific Events, p.13, 11-14.08.2025, Burgas, Bulgaria, authors: Enculescu M., Nicolae I., Gavojdian D.)

The aim of this research was to evaluate the effects of Nigella sativa oil dietary supplementation on methane (CH₄) emissions in lactating dairy cows. The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, between December 2024 and March 2025, on 40 Romanian Black and Spotted dairy cows, divided into two groups: the Experimental group (E: n=20) and the Control group (C: n=20). The E group received 10 ml of Nigella sativa oil/head/day for 90 consecutive days. The methane concentrations were measured using a methane detector (Portable Multi-Gas Monitor GX-6000, Riken Keiki, Tokyo, Japan) at the following intervals: day - 0 (t₀), 30 days (t₁), 60 days (t₂), and at 90 days (t₃). The methane detector was pointed directly towards the cows' right nostril (45° angle) at a distance of 1 m. The measurement length was 5 minutes/cow/day, between 10:00 AM and 14:00 PM, for 4 consecutive days/interval. A t-test was used to evaluate the influence of Nigella sativa oil addition on the methane emissions. The results showed significant differences for the E group compared with the C group (E: 2.092±188 ppm vs. C: 2.611±112 ppm, P=0.024) at the end of the experiment (T₃). The results suggest that the addition of Nigella sativa oil to dairy cows' diets could lead to a reduction in CH₄ emissions.

Table 2. Results of Nigella sativa oil influence on methane emissions in dairy cattle

Specifications	X±SEM	SD	CV	Min	Max	E vs. C
E-T ₀	2.125±184	822	38.69	125	3.500	T ₀ : P=0.835
C-T ₀	2.063±233	1.044	50.61	250	4.250	

E-T ₁	2.291±351	1.568	68.44	188	5.438	T_1 : P=0.451
C-T ₁	2.617±246	1.098	41.97	125	3.815	
E-T ₂	1.994±316	1.412	70.80	500	4.625	T ₂ : P=0.274
C-T ₂	2.453±267	1.195	48.71	500	4.500	
E-T ₃	2.092±188	839	40.10	750	4.125	T ₃ : P=0.024
C-T ₃	2.611±112	500	19.14	1.375	3.625	

V. TACKLING WEANING STRESS IN DAIRY CALVES USING CANNABIDIOL OIL SUPPLEMENTATION-A PILOT STUDY (article published in Dairy Journal, 6(5):54, 2025, https://doi.org/10.3390/dairy6050054, authors: Enculescu M., Nicolae I., Gavojdian D.)

This pilot study evaluated the effects of cannabidiol (CBD) oil supplementation on growth performance, stress biomarkers, and haematological profiles in dairy calves undergoing the weaning transition. Nineteen Holstein calves were divided into two paternal-sibling groups: a CBD-supplemented experimental group (n = 10) and a CON-control group (n = 9).

The CBD group received 5 ml/head/day of CBD oil for the first two days (pre-weaning), followed by 10 ml/head/day for three consecutive days post-weaning. Body weight increased significantly over time in both groups (p = 0.000), nevertheless no significant differences were observed between groups (p = 0.173) or for the group × time interaction (p = 0.929), indicating that CBD did not affect overall growth trajectory. However, a significant group \times time interaction (p = 0.006) for average daily gains in the CBD group was observed. Serum cortisol concentrations were significantly lower in CBD-supplemented calves at Day 0 and +2 days, compared to the CON group, indicating a transient anti-stress effect (p = 0.043 for group effect). At +5 days, cortisol levels in the CBD group increased, surpassing control values, though this difference was not significant. A trend-level group × time interaction (p = 0.067) suggested a distinct temporal cortisol response in CBD-treated calves. Immune cell counts (LYM, MON, NEU) showed no significant differences, though monocyte levels trended lower in CBD calves at early time points. Platelet indices revealed a significant reduction in mean platelet volume (p = 0.047) and stable PDWc and platelets values in the CBD group, suggesting modulation of inflammatory status. Alanine aminotransferase levels increased over time with a significant group effect (p = 0.014), indicating a mild hepatic response, while glucose and alkaline phosphatase remained within physiological ranges.

These findings suggest that short-term CBD supplementation may transiently modulate stress and inflammatory responses during weaning, with potential benefits for physiological resilience. However, rebound endocrine effects and hepatic sensitivity highlight the need for further research to refine dosing strategies and assess long-term safety in dairy production systems.

Table 3. Effect of CBD oil on the body weight (kg) in dairy calves

Variable	Group	-7 days ⁴	Day 0 ⁵	+7 days ⁶	+14 days ⁷	+21days ⁸
		Mean ±	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
		SEM ⁹				
	CBD ²	94.00 ±	95.00±1.86 ^{BC}	104.60±2.87 ^{AB}	109.80±3.38 ^A	114.20±3.97 ^A
		2.41 [°]				
BW^1	CON ³	96.89±	102.33±4.52 ^{BC}	107.44±4.20 ^{AB}	111.67±3.40 ^A	115.22±4.39 ^A
		4.84 ^C				
	Effe	ect				
	Group	p=0.173				
	Time	p=0.000				
	Group x	p=0.929				
	Time					

 $^{1}BW=$ body weight; ^{2}CBD group = experimental group supplemented with cannabidiol (CBD) oil; ^{3}CON group = non-supplemented control group; 4 -7 days = days before weaning; ^{5}Day 0 = day of weaning; 6 +7 days = days after weaning; 7 +14 days = days after weaning; 8 +21 days = days after weaning; ^{9}SEM = standard error of the mean; Statistical significance: p < 0.05, and trends $p \le 0.10$; Means that do not share a letter are significantly different.

Figure 12. Immune response timeline comparing the CBD group and the CON group. The NEU/LYM ratio illustrates differential immune stress and recovery profiles at sampling times

Day 0, +2 days, and +5 days

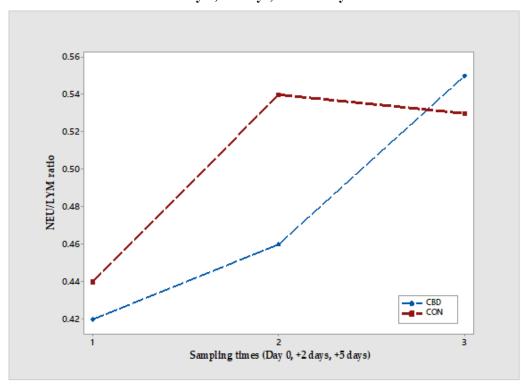


Table 4. Effect of CBD oil on GLU (mg/dl), ALP (IU/L), and ALT (IU/L) indicators in dairy calves

Variable	Group	Day 0 ⁶	+2 days ⁷	+5 days ⁸	Effect		
		Mean ±	Mean ±	Mean ±	Group	Time	Group x
		SEM ⁹	SEM	SEM			Time
GLU ¹	CBD ⁴	104.90±3.53 ^A	105.89±3.23 ^A	97.00±5.45 ^B	p=0.348	p=0.039	p=0.845
	CON ⁵	109.67±2.46 ^A	106.44±2.93 ^B	100.11±2.74 ^B			
ALP ²	CBD	291.0±16.0 ^A	322.8±27.8 ^A	297.9±30.4 ^A	p=0.213	p=0.778	p=0.847
	CON	268.9±31.5 ^A	276.4±27.4 ^A	281.2±28.1 ^A			
ALT ³	CBD	8.30±0.49 ^A	10.60±0.74 ^A	11.30±0.87 ^B	p=0.014	p=0.000	p=0.358
	CON	8.88±0.79 ^A	13.33±0.91 ^A	12.66±0.60 ^B			

 $^{{}^{7}}GLU = glucose; {}^{2}ALP = alkaline \ phosphatase; {}^{3}ALT = alanine \ aminotransferase; {}^{4}CBD \ group = experimental \ group \ supplemented \ with \ cannabidiol \ (CBD) \ oil; {}^{5}CON \ group = non-supplemented \ control \ group; {}^{6}Day \ 0 = day \ of \ weaning; {}^{7}+2 \ days = two \ days \ post-weaning; {}^{8}+5 \ days = five \ days \ post-weaning; {}^{9}SEM = standard \ error \ of \ the \ mean; \ Statistical \ significance: <math>p < 0.05$, and trends $p \le 0.10$; Means that do not share a letter are significantly different.

VI. <u>INTEGRATING MULTI-MODAL DATA FUSION APPROACHES FOR ANALYSIS OF</u>

<u>DAIRY CATTLE VOCALIZATIONS</u> (article published in Frontiers in Veterinary Science Journal, 12, 2025, p.1704031, https://doi.org/10.3389/fvets.2025.1704031, authors: Jobarteh B., Mincu-Iorga M., Gavojdian D., Neethirajan S.)

Non-invasive analysis of dairy cattle vocalizations offers a practical route to continuous assessment of stress and timely health interventions in precision livestock systems. We present a multimodal AI framework that fuses standard acoustic features (e.g., frequency, duration, amplitude) with non-linguistic, transformer-based representations of call structure for behavior classification. The classification analysis represents the core contribution of this work, while the integration of the Whisper model serves as a complementary exploratory tool, highlighting its potential for future motif-based behavioral studies. Using contact calls recorded from a cohort of lactating Romanian Holsteins during a standardized, brief social-isolation paradigm, we developed an ontology distinguishing high-frequency calls (HFCs) associated with arousal from low-frequency calls (LFCs) associated with calmer states. Across cross-validated models, support vector machine and random-forest classifiers reliably separated call types, and fused acoustic + symbolic features consistently outperformed single-modality inputs. Feature-importance analyses highlighted frequency, loudness, and duration as dominant, interpretable predictors, aligning vocal patterns with established markers of arousal. From a clinical perspective, the system is designed to operate passively on barn audio to flag rising stress signatures in real time, enabling targeted checks, husbandry adjustments, and prioritization for veterinary examination. Integrated with existing sensor networks (e.g., milking robots, environmental monitors), these alerts can function as an early-warning layer that complements conventional surveillance for conditions where vocal changes may accompany pain, respiratory compromise, or maladaptive stress. While the present work validates behaviorally anchored discrimination, ongoing efforts will pair vocal alerts with physiological measures (e.g., cortisol, infrared thermography) and multi-site datasets to strengthen disease-specific inference and generalizability.

This framework supports scalable, on-farm welfare surveillance and earlier intervention in emerging health and stress events. This study presents a novel multi-modal framework for analyzing cattle vocalizations, integrating acoustic and symbolic features within machine learning classifiers to advance automated behavior classification and real-time welfare monitoring in precision livestock systems. Our hypothesis was that fusing standard acoustic features with Whisper-derived symbolic motifs improves discrimination between high-frequency (HF) and low-frequency (LF) calls compared with single-modality models (acoustic-only or symbolic-only).

We formulated the following predictions: (i) model hierarchy: SVM ≥ Random Forest > RNN under our data constraints; (ii) top features: frequency, loudness, and duration will rank highest; (iii) motifs: frequent bigrams (e.g., "rr") will align with HFC episodes; and (iv) performance: fused features will outperform either modality alone.

Table 5. Performance Evaluation Result of Random Forest, Support Vector Machine and RNN

Model	Class	Precision	Recall	F1-Score	Accuracy
Random	HFC	0.98	0.99	0.98	0.9725
Forest	пгс	0.98	0.99	0.98	0.9723
	LFC	0.95	0.93	0.94	
	Macro Avg	0.97	0.96	0.96	
	Weighted Avg	0.97	0.97	0.97	
SVM	HFC	0.99	0.99	0.99	0.9835
	LFC	0.98	0.96	0.97	
	Macro Avg	0.98	0.97	0.98	
	Weighted Avg	0.98	0.98	0.98	
RNN	HFC	0.89	0.97	0.93	0.88
	LFC	0.88	0.62	0.73	
	Macro Avg	0.88	0.80	0.83	
	Weighted Avg	0.88	0.88	0.88	

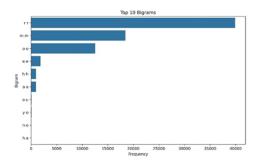




Figure 13. Bigram Frequency Analysis of symbolic cow vocalizations (left side); Word Cloud of bigram frequencies, where larger text size reflects higher occurrence, with 'rr' and 'mm' as dominant patterns (right side)

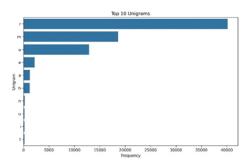




Figure 14. Left side – Unigram Frequency Distribution, showing 'r', 'm', and 'o' as the most frequent characters, indicating consistent symbolic encoding of common vocal elements; Right side – Word Cloud of unigram frequencies, highlighting dominant vocal elements in symbolic form

VII. <u>BEYOND YIELD: AN INTEGRATED ANALYSIS OF PERFORMANCE, HEALTH AND BEHAVIOUR IN ROMANIAN DUAL-PURPOSE CATTLE BREEDS</u> (article accepted for publication in the journal Revista Română de Medicină Veterinară, vol. 35:4/2025, authors: Minculorga M., Mizeranschi A.E., Gavojdian D., Nicolae I., Enculescu M., Ilie D.E.)

This study compared milk production traits in two Romanian dual-purpose cattle breeds, the Romanian Spotted (SIM) and the Romanian Brown (BSW), using integrated phenotypic, behavioural and genomic data from 232 cows. We evaluated test-day milk yield, fat and protein content, somatic cell score (SCS), milking reactivity, body condition score (BCS) and animal-based welfare indicators. Genotyping was performed using 88 single nucleotide polymorphisms (SNPs) linked to temperament and milking behaviour. Results showed that BSW cows produced significantly more milk but had higher SCS, suggesting a productivity-health trade-off. Milking reactivity had a non-linear effect on both yield and SCS, while parity and BCS also influenced performance. Correlation analysis highlighted breed-specific trade-offs between yield and milk composition. Genomic analysis confirmed distinct population structures between the breeds, with greater variability observed in SIM cows. Overall, current findings

support the integration of behavioural and genomic data into dual-purpose breed evaluation to improve selection and veterinary management strategies in dairy systems.

Table 6. Mean ± SEM for milk yield, fat, protein and somatic cells score (SCS) in Romanian Spotted cows (SIM) and Romanian Brown cows (BSW)

Breed	Milk yield	Fat	Protein	SCS
Indicators	[kg/test day]	[%/test day]	[%/test day]	[cells/mL]
Cohort	17.11 ± 0.40	3.51 ± 0.05	3.50 ± 0.03	4.23 ± 0.13
SIM (n=185)	16.94 ± 0.44	3.48 ± 0.05	3.49 ± 0.03	3.98 ± 0.14
BSW (n=47)	17.77 ± 0.89	3.63 ± 0.13	3.53 ± 0.06	5.19 ± 0.30

Table 7. Summary of linear regression model outputs for milk production and health traits in Romanian Spotted cows (SIM) and Romanian Brown cows (BSW)

redictor	Milk Yield (kg/day)	Fat (%)	Protein (%)	SCS
ntercept	3.63 ± SE***	3.32 ± SE***	3.06 ± SE***	3.63 ± SE***
reed (ref: BSW)				
IM	-1.29 ± SE***	$-0.17 \pm SE$	$-0.037 \pm SE$	-1.2918 ± SE***
arity	0.27 ± SE***	-0.02 ± SE	$0.0014 \pm SE$	0.2654 ± SE***
ays in milk (DIM)	$0.002 \pm SE*$	0.00157 ± SE***	0.00177 ± SE***	$0.0017 \pm SE*$
lilking Reactivity				1
inear	$0.18 \pm SE$	-	-0.108 ± SE	-
uadratic	$-0.29 \pm SE$	-	-	-
ubic	$0.06 \pm SE$	-	-	-
uartic	-0.72 ± SE**	-	-	-0.7214 ± SE**
CS (ref: Optimal)			ı	1
ean (Score 1)	$0.46 \pm SE$	$0.210 \pm SE$	$0.108 \pm SE$	$0.190 \pm SE$
bese (Score 2)	0.84 ± SE**	$0.039 \pm SE$	$0.000 \pm SE$	0.8439 ± SE**
dder cleanliness	NS	NS	NS	NS
arsal joint lesions	NS	NS	NS	NS
Claw overgrowth	NS	NS	NS	NS
djusted R ²	0.2185	0.0910	0.3093	0.1906
Todel p-value	< 0.001	0.0009	< 0.001	< 0.001
law overgrowth	NS 0.2185	NS 0.0910	0.3093	NS 0.1906

Values are presented as estimate \pm Standard Error (SE). Significance levels: *p < 0.05, **p < 0.01, **p < 0.001. NS = Not Significant. Dashes (-) indicate non-significant

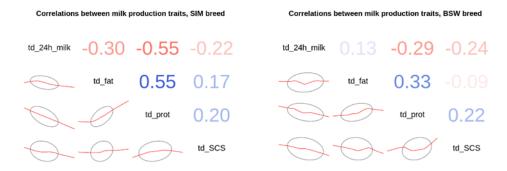


Figure 15. Correlation matrix of milk production traits in Romanian Spotted, SIM (a) and Romanian Brown, BSW (b) breeds. The intensity of the colour and size of the values indicate the strength of the correlation, with red representing negative and blue representing positive relationships; *td=test day

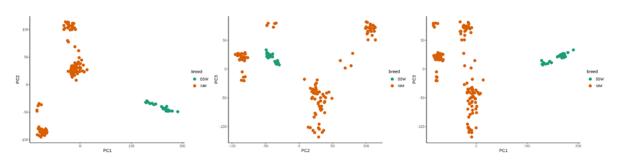


Figure 16. Population structure from the principal component analysis of the 88 SNPs.

Population structure is presented as pairwise scatter plots (a–c) of the first three principal components (PC), with green and orange dots representing the two breeds (Romanian Spotted – SIM in orange and Romanian Brown – BSW in green). (a) PC1 vs. PC2; (b) PC2 vs. PC3; (c) PC1 vs. PC3

DISSEMINATION OF RESULTS:

Scientific articles:

Jobarteh B., Mincu-Iorga M., Gavojdian D., Neethirajan S., 2025, Integrating Multi-Modal Data Fusion Approaches for Analysis of Dairy Cattle Vocalizations, *Frontiers in Veterinary Science*, 12, p.1704031, https://doi.org/10.3389/fvets.2025.1704031 (ISSN 2297-1769, journal impact factor: 2.9, ranked Q1 in the Web of Science category '*Veterinary Sciences*');

Enculescu M., Nicolae I., Gavojdian D., 2025, Tackling Weaning Stress in Dairy Calves Using Cannabidiol Oil Supplementation-A Pilot Study, *Dairy*, 6(5):54, https://doi.org/10.3390/dairy6050054 (ISSN 2624-862X, journal impact factor: 3.1, ranked Q1 in the Web of Science category '*Agriculture*, *Dairy and Animal Science*');

Mincu-Iorga M., Mizeranschi A.E., Gavojdian D., Nicolae I., Enculescu M., Ilie D.E., 2025, Beyond yield: an integrated analysis of performance, health and behaviour in Romanian dual-purpose cattle breeds, *Revista Română de Medicină Veterinară*, article accepted for publication, vol.

35:4/2025, (ISSN 1220-3173, journal impact factor: 0.2, ranked Q4 in the Web of Science category 'Veterinary Sciences').

Attending conferences:

- Mincu-Iorga M., Gavojdian D., Machine learning-based analysis of dairy cow vocal behaviour, *Book of abstract of the International Scientific Symposium "Young People and Multidisciplinary Research in Applied Life Sciences"* ISSN: 2821-4285, ISSN L: 2821-4285 p. 47, 13-14.11.2025, Timişoara, (poster presentation), https://www.usab-tm.ro/en/scientific-symposium-85/symposium;
- Mincu-Iorga M., Mizeranschi A.E., Gavojdian D., Nicolae I., Ilie D.E., Genome-Wide Association Studies for Milking Reactivity in Romanian Spotted Cattle, *Book of Abstracts of the 76th European Federation of Animal Science*, ISBN: 979-12-210-6769-9 vol. 39, p. 777, 25-29.08.2025, Innsbruck, Austria, (poster presentation), https://eaap2025.org/;
- Enculescu M., Nicolae I., Gavojdian D., Effect of Nigella sativa oil on methane emissions in dairy cattle, *13th International Conference, Agriculture & Food 2025, International Scientific Events,* p.13, 11-14.08.2025, Burgas, Bulgaria, (poster presentation), <u>www.sciencebg.net</u>;
- Mincu-Iorga M., Gavojdian D., Nicolae I., Zamansky A., Lazebnik T., AI approaches for detection and removal of environmental sounds in audio recordings to enhance automated annotation and extraction of cattle vocal parameters, *Proceedings of the 58th Congress of the International Society for Applied Ethology (ISAE)*, p. 400, 4-8.08.2025, Utrecht, The Netherlands, (poster presentation), https://isae2025utrecht.nl/.

FINAL TABLE OF INDICATORS FOR STAGE 1/2025

No.	Stage Activity Title Involved Partner	Verifiable Estimated Results Activity Category Partner Activities		
1	Stage 1 – Strengthening the institutional research capacity of ICDCB Balotesti	Dissemination of project results through participation in international conferences; Publication of scientific articles in Web of Science journals	Number of minimum indicators assumed	Indicators Achieved Stage 1/2025
2	Act 1.1 – Participation in international conferences	D – Support Activities (Participation in scientific-technical events; Work visits)	Participation in 2 international conferences with	Participation in 4 international conferences with
3	1.1.1 – Partner – Coordinator (CO) – RESEARCH AND DEVELOPMENT INSTITUTE FOR BOVINE BALOTESTI	Participation in prestigious international conferences in the field of bovine science	presentation of results	presentation of results
4	Act 1.2 – Publication of scientific articles in Web of Science journals	D – Support Activities (Participation in scientific-technical events; Work visits)	Publication of 2 articles in Web of Science journals	Publication of 3* articles in Web of Science journals
5	1.2.1 – Partner – Coordinator (CO) – RESEARCH AND DEVELOPMENT INSTITUTE FOR BOVINE BALOTEȘTI	Submission of articles for publication in prestigious journals in the field of bovine science		, and the second
			-	Submission of a Romania–France bilateral project proposal (2025 call)

^{*} An article has been accepted for publication in the Revista Română de Medicină Veterinară and is scheduled to be published in Vol. 35, No. 4/2025, in December 2025

<u>Interim Scientific Report, project code PN-IV-P8-8.1-PRE-HE-ORG-2025-0265, implementation 14.07-31.12.2025</u>

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