

BOOK OF ABSTRACTS

2026 IEEE INTERNATIONAL WORKSHOP ON

Measurements and Applications in Veterinary and Animal Sciences

PADOVA - APRIL 28-30, 2026

MeAVeAS PADOVA 2026

IEEE

Università degli Studi di Padova

WORKSHOP PROGRAM

Tuesday, April 28

S1.1 - Bridging sustainability and intrinsic quality of animal-sourced foods: conceptual frameworks, tools and metrics

Room: Sala Luna

1 **Sustainability Assessment of Small Ruminant Farming System in Northern Apulia Using the FAO SAFA Framework**

Antonella della Malva, Emilia Lamonaca, Maria Giovanna Ciliberti, Antonella Santillo and Marzia Albenzio (University of Foggia, Italy)

2 **Advancing Nutritional Life Cycle Assessment (nLCA): Evaluating Functional Units for Integrating Nutrition, Health, and Environmental Impact of Animal Sourced Foods**

Lynda Perkins (The Queen's University Belfast, United Kingdom (Great Britain)); Ryan Mcguire, Lana Bothwell and Anne Nugent (Queen's University Belfast, United Kingdom (Great Britain)); Mohammed Gagaoua (PEGASE, INRAE, Institut Agro, France); Nigel Scollan (Queen's University Belfast, United Kingdom (Great Britain))

4 **Direct Prediction of Beef Eating Quality from Raw Muscle Using REIMS Spectral Output**

Lynda Perkins (The Queen's University Belfast, United Kingdom (Great Britain)); Wenyang Jia (Queen's University Belfast, United Kingdom (Great Britain)); Alix Neveu (Birkenwood Pty Ltd, Australia); Tim Rowe (Castell Howell Foods Ltd and Celtica Foods Ltd, United Kingdom (Great Britain)); Pip Nicholas-Davies (Aberystwyth University, United Kingdom (Great Britain)); Anastasios Koidis Koidis (Queens University Belfast, United Kingdom (Great Britain)); Nigel Scollan (Queen's University Belfast, United Kingdom (Great Britain))

6 **How Do Conventional, Organic and Free-Range Pig Farming Systems Differ in Their Environmental Impacts? A Meta-Analysis and Multivariate Analysis of Pig Life Cycle Assessment Studies**

Mohammed Gagaoua (PEGASE, INRAE, Institut Agro, France); Adrien Karolak (PEGASE, INRAE, Institut Agro, Saint-Gilles, France)

8 **Development and Validation of Portable NIRS Calibration Models for Predicting Fecal Composition**

Chiara Evangelista and Federico Manganello (University of Tuscia, Italy); Francesca Petrocchi Jasinski (University of Tuscia, Viterbo, Italy); Loredana Basiricò, Umberto Bernabucci and Pier Paolo Danieli (University of Tuscia, Italy)

S1.2 - Metrology for Food Quality, Safety, and Traceability - PART I

Room: Sala Europa

9 **From Benchtop to Portable Sensors: NIR Spectroscopy and Colour Analysis for Honey Quality and Origin Evaluation**

Alessia Zoroaster (University of Padua, Italy); Andrea Calore, Severino Segato, Paolo Berzaghi and Anisseh Sobhani (University of Padova, Italy); Nicoletta Dainese (Istituto Zooprofilattico Sperimentale delle Venezie, Italy); Lorenzo Serva (University of Padua, Italy)

10 **Critical Importance of pH and Aw Measurement Site in Dry-Aged Meat and Its Implications for Food Safety**

Federica Savini (University of Bologna, Italy); Elena Zanato (University of Bologna); Giulia Polizzi, Laura Prandini, Alessandro Seguino, Alessandra De Cesare and Valentina Indio (University of Bologna, Italy); Silvia Vianello and Lucia Buratti (Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Bologna, Italy); Andrea Serraino (University of Bologna, Italy)

12 **Virulence Potential and Antimicrobial Resistance of Bacillus Cereus Isolated from Infant Formulae**
Viviana Fusi (University of Milan, Italy); Simone Stella (Università degli Studi di Milano, Italy); Emilia Ghelardi and Francesco Celandroni (Università di Pisa, Italy); Cristian Bernardi, Filippa Maria Addis and Clara Locatelli (Università degli Studi di Milano, Italy); Christian Scarano, Francesca Piras and Giuliana Siddi (Università Degli Studi di Sassari, Italy); Erica Tirloni (Università degli Studi di Milano, Italy)

13 **Dairy Cows Affected by Intramammary Infection, Subclinical and Clinical Mastitis: Changes in Milk Metabolome Profiles**
Lisuzzo Anastasia (University of Padua, Legnaro (PD), Italy); Maria Chiara Alterisio and Sergio Esposito (University Federico II Naples, Italy); Luca Laghi (University of Bologna, Italy); Antonella Pesce (Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy); Paolo Ciaramella (University Federico II Naples, Italy); Francesca Cecchini (University of Padua (PD), Italy); Giorgia Taio (University of Padua, Italy); Michele Berlanda and Matteo Gianesella (University of Padua (PD), Italy); Jacopo Guccione (University Federico II Naples, Italy); Enrico Fiore (University of Padua, Legnaro (PD), Italy)

S1.3 - Advanced dissuasion techniques and technologies for the protection of livestock against predations

Room: Sala Ganimede

14 **An Integrated Non-Lethal Deterrence Solution Approach to Mitigate Wolf-Livestock Conflicts**
Leonardo Martin, Giacomo Peruzzi and Matteo Bertocco (University of Padova, Italy); Nicola Trivellin (UNIPD, Italy); Alessandro Pozzebon (University of Padova, Italy)

15 **A Dual Triggered Smart Detection System Based on RADAR and PIR for Wolf-Livestock Conflict Mitigation**
Leonardo Martin, Giacomo Peruzzi and Matteo Bertocco (University of Padova, Italy); Nicola Trivellin (UNIPD, Italy); Alessandro Pozzebon (University of Padova, Italy)

16 **Field Testing of an Acoustic anti-Wolf Collar for Livestock Protection in Southern Italy**
Pietro Orlando (University of Reggio Calabria, Italy)

17 **Molecular Measurement Workflows for Tick-Borne Pathogen Detection in Sub-Saharan Livestock**
Tingxi Zhang and Marika Grillini (University of Padua, Italy); Giorgia Dotto (University of Padova, Italy); Eric Yessinou (University of Abomey-Calavi, Benin); Hermogenes Neves Mucache and Luis Neves (Eduardo Mondlane University, Mozambique); Rudi Cassini (Unievrsity of Padova, Italy)

S2.1 - Prototype Systems and Measurement Technologies for Assessing Behavioural Patterns in Livestock

Room: Sala Luna

18 **A Wearable Vibration System for Communicating with Dairy Cows**
Hen Honig (Department of Animal Welfare, Israel); O. Forkosh (Hebrew University of Jerusalem, Israel); Y. Salzer, B. Shaked, L. Rosenfeld, G. Lidor, L. Reshef and N. Saraf (Agricultural Research Organization, Israel)

19 **Sensor-Based Detection of Drinking Behaviour and Water Intake Variability in Dairy Cattle for Precision Farming**
Rebecca Goretti, Damiano Cavallini, Jacopo Vegni, Martina Lamanna, Andrea Formigoni, Riccardo Colleluori, Marco Bovo, Carlos Alejandro Perez Garcia, Giulio Visentin, Yari Vecchio and Angela Costa (University of Bologna, Italy)

21 **Does Feed Anticipation Modulate Vocal Acoustic Parameters in Young Water-Buffaloes?**
Madalina Mincu-Iorga and Dinu Gavojdian (Research and Development Institute for Bovine, Romania)

22 **Water-to-Gain Balance Identifies Distinct Adaptation Trajectories in Young Beef Cattle Imported from France**
Caterina Marcon (University of Padua, Italy); Alessia Visentini, Simone Dante and Luisa Magrin (University of Padova, Italy)

23 **High-Resolution Monitoring of Social Interactions in Dairy Cows Using a Prototype Ultra-Wideband System**
Valentina Becciolini, Sebastian Schweizer and Matteo Barbari (University of Florence, Italy)

S2.2 - Metrology for Food Quality, Safety, and Traceability - PART II

Room: Sala Europa

24 **Exploring Natural Antimicrobial Strategies Against Campylobacter Spp. with Diverse Antimicrobial Resistance Profiles Along the Poultry Production Chain**

Rossana Roila and Andrea Cantarini (University of Perugia, Italy); Francesca Piras and Christian Scarano (Università Degli Studi di Sassari, Italy); Enrico De Santis (University of Rome La Sapienza, Italy); Erica Tirloni, Simone Stella and Filippa Maria Addis (Università degli Studi di Milano, Italy); Giuliana Siddi (Università Degli Studi di Sassari, Italy); David Ranucci, Rana Karasoy and Raffaella Branciari (University of Perugia, Italy)

26 **Development of a Spectral Library for the Detection of Nitrite Fraud in Tuna**

Martina Marcolin (University of Padua, Italy); Sarah Currò (Università Degli Studi di Padova, Italy); Tiziana Nardin (Fondazione Edmund Mach, Italy); Enrico Novelli, Luca Fasolato and Stefania Balzan (Università Degli Studi di Padova, Italy)

27 **Inactivation of Opistorchis Felineus Metacercariae in Tench by Freezing and Combined Treatment with Smoking and Refrigeration**

Raffaella Franceschini (University Tor Vergata, Rome, Italy); Francesco Agnetti (IZSUM, Italy); David Ranucci, Rossana Roila, Rana Karasoy, Andrea Cantarini and Raffaella Branciari (University of Perugia, Italy)

28 **Characterization of Goat Milk Cheeses from Native Breeds Produced Using Traditional and Innovative Ripening Methods**

Marika Di Paolo and Marica Egidio (University of Naples Federico II, Italy); Rosa Luisa Ambrosio (University Federico II Naples, Italy); Federica Capano (University of Federico II of Naples, Italy); Michelangelo Galella (ASP Potenza, Department of Prevention, Italy); Loriana Casalino (Universitas Mercatorum, Italy); Salvatore Claps (CREA Research Centre for Animal Production and Aquaculture, Italy); Raffaele Marrone (University of Naples Federico II, Italy)

29 **Application of Electronic Nose Technology to Differentiate Aroma Profiles in Bovine and Buffalo Cheeses Subjected to Various Dietary Treatments**

Loriana Casalino (Universitas Mercatorum, Italy); Marika Di Paolo, Marica Egidio, Sara Balestra and Giovanmaria Pacelli (University of Naples Federico II, Italy); Anna Balestrieri (Istituto Zooprofilattico del Mezzogiorno, Italy); Raffaele Marrone (University of Naples Federico II, Italy)

30 **Repeatability of MALDI-TOF MS Campylobacter Jejuni Spectra in Sub-Species Studies**

Evgeniia Igosheva (University of Padua, Italy); Laura Prandini, Valentina Indio, Federica Savini and Alessandra De Cesare (University of Bologna, Italy); Roberta Tolosi (University of Padua, Italy); Andrea Serraino (University of Bologna, Italy); Federica Giacometti (University of Padova, Italy)

S2.3 - AI applications in Veterinary Medical Imaging

Room: Sala Ganimede

31 **The Longissimus Dorsi Ultrasound Evaluations Influenced by Stocking Density in Beef Heifers**

Lisuzzo Anastasia (University of Padua, Legnaro (PD), Italy); Giorgia Taio (University of Padua, Italy); Silvia Miretti (University of Torino, Italy); Carmine Versace (University of Turin, Grugliasco, Italy); Paolo Cornale and Antonio Mimosi (University of Turin, Italy); Enrico Fiore (University of Padua, Legnaro (PD), Italy); Francesca Cecchini (University of Padua (PD), Italy); Chiara Tommasoni (University of Padua, Italy); Matteo Boso (Veterinary Practitioners, Italy); Matteo Gianesella (University of Padua (PD), Italy)

32 **UNet for Automatic Pneumothorax Detection on Canine and Feline CTs**

Nicolò Mastromattei (University of Padua, Italy); Artur Jurgas (AGH University of Krakow, Poland); Tommaso Pilla (Animal Hospital Postojna, Italy); Diane Wilson (Antech Imaging Services, USA); Marek Wodzinski (AGH University of Krakow, Poland); Andre Dourson (Antech Imaging Services, USA); Silvia Burti, Polina Zemko and Alessandro Zotti (University of Padua, Italy); Tommaso Banzato (University of Padova, Italy)

33 **Deep Learning-Based Automated Segmentation of Canine Adrenal Glands Nodules on CT-Images**

Silvia Burti and Nicolò Mastromattei (University of Padua, Italy); Artur Jurgas and Marek Wodzinski (AGH University of Krakow, Poland); Diane Wilson (Antech Imaging Services, USA); Gaia De Lorenzi, Nicole Antonella Lotesoriere and Alessandro Zotti (University of Padua, Italy); Tommaso Banzato (University of Padova, Italy)

34 **Development of an Artificial Intelligence-Based Method for the Quantification of Left Atrial Size in Dogs from Lateral Thoracic Radiographs**
Carlotta Valente (University of Padua, Italy); Marek Wodzinski (AGH University of Krakow, Poland); Leonardo Galvan, Chiara Gerardo and Carlo Guglielmini (Dept. of Animal Medicine Production and Health University of Padua Legnaro PD, Italy); Helen Poser (Università di Padova, Italy); Alessandro Zotti (University of Padua, Italy); Tommaso Banzato (University of Padova, Italy)

35 **Benchmarking Deep Architectures for Automated VLAS Measurement in Canine Lateral Thoracic Radiographs**
Marek Wodzinski (AGH University of Krakow, Poland); Carlotta Valente (University of Padua, Italy); Artur Jurgas (AGH University of Krakow, Poland); Leonardo Galvan, Chiara Gerardo and Carlo Guglielmini (Dept. of Animal Medicine Production and Health University of Padua Legnaro PD, Italy); Helen Poser (Università di Padova, Italy); Alessandro Zotti (University of Padua, Italy); Tommaso Banzato (University of Padova, Italy)

36 **Region-Based Hierarchical Training for Nested Small-Structure Segmentation in Veterinary CT**
Artur Jurgas (AGH University of Krakow, Poland); Silvia Burti and Nicolò Mastromattei (University of Padua, Italy); Marek Wodzinski (AGH University of Krakow, Poland); Gaia De Lorenzi and Nicole Antonella Lotesoriere (University of Padua, Italy); Tommaso Banzato (University of Padova, Italy)

Wednesday, April 29

S3.1 - Smart sensing and computer vision applications for monitoring animal behaviour, health, and welfare - PART I
Room: Sala Luna

37 **Open-Set Recognition of Dairy Cows with Computer Vision and RFID**
Claudia Giannone, Carlos Alejandro Perez Garcia, Damiano Cavallini, Martina Lamanna, Marco Bovo, Andrea Formigoni, Daniele Torreggiani and Patrizia Tassinari (University of Bologna, Italy)

38 **The Importance of Having Enough Space: Assessing Lying Behaviour in Horses Housed in Single Boxes**
Chiara Maccario (Università degli Studi di Milano, Italy); Elie Atallah and Emanuela Dalla Costa (Università Degli Studi di Milano, Italy)

39 **What We Know About Tools in Horse Sleep/Resting Behavior and Why We Should Ask More**
Angela Perrone Barbosa, Tiago Marcelo Oliveira, Roberta Carvalho Basile and Raquel Yvonne Arantes Baccarin (Universidade de São Paulo, Brazil)

40 **How Does Social Reshuffling Influence Lying Behaviour in Donkeys?**
Elie Atallah (Università Degli Studi di Milano, Italy); Chiara Maccario (Università degli Studi di Milano, Italy); Elisa Basso Ricci and Emanuela Dalla Costa (Università Degli Studi di Milano, Italy)

41 **Semi-Automated Assessment of the Ecotoxicological Effects of 3D Printing Materials on the Survival and Reproduction of Folsomia Candida**
Anita Casadei (Scuola Superiore Sant'Anna, Italy); Lorenzo Filicetti and Antonio DeSimone (Sant'Anna School of Advanced Studies, Italy); Donato Romano (Scuola Superiore Sant Anna, Italy)

43 **Tech-Driven Evolution of Animal Housing: Exploring Technologi-Cal Integration and Challenges in Mountain Livestock Farming**
Andrea Silvestri and Monica Parlato (University of Padua, Italy); Andrea Pezzuolo (University of Padova, Italy)

S3.2 - Biologging for Animal Health, Welfare and Environmental Adaptation
Room: Sala Europa

44 **Circadian Collapse-Recovery Dynamics of Physiological and Behavioral Signals Improve Estrus Prediction in Sheep Using Wearable Sensors and AI-Based Modeling**
José-Alfonso Abecia and Adriana Casao (Universidad de Zaragoza, Spain); María Pilar Pina (UNIZAR, Spain); Marta Lafuente (UPNA, Spain); Cristina Peña, Ricardo López and Ignacio Ontañón (UNIZAR, Spain); Rosaura Pérez-Pe (Universidad de Zaragoza, Spain)

45 **Autonomic Dynamics and Early Prediction of Parturition in Sheep Using Continuous Physiological Signals**
Carlos Palacios, Jaime Nieto and Javier Plaza (Universidad de Salamanca, Spain); José-Alfonso Abecia (Universidad de Zaragoza, Spain)

46 **Use of Bio-Loggers to Assess Heat Stress in Piedmontese Cows Under Different Stocking Densities**
Isabella Manenti (Universita Degli Studi Torino, Italy); Giovanni Perona (University of Turin, Italy); Stefano Di Carlo (Politecnico of Turin, Italy); Roberta Bardini, Lorenzo Martini and Alessia Leclercq (Politecnico di Torino, Italy); José-Alfonso Abecia (Universidad de Zaragoza, Spain); Asgeir Bjarnason (Star-Oddi & Star-Oddi Ltd., Iceland); Silvia Miretti (University of Torino, Italy); Paolo Cornale (University of Turin, Italy)

47 **The Connection Between Heart Rate, Accelerometry, and Metabolic Rate in Ungulates: Physiological Meaning and Applications for Biologging**
Asgeir Bjarnason (Star-Oddi & Star-Oddi Ltd., Iceland)

48 **Cardiac Dynamics of Mares During Different Breeding Procedures: Mixed-Effects Models of HR and HRV Using Polar Sensors**
Javier Plaza (Universidad de Salamanca, Spain); Santos Del Pozo and María Elena Pérez García (University of Salamanca, Spain); Jaime Nieto (Universidad de Salamanca, Spain); José-Alfonso Abecia (Universidad de Zaragoza, Spain); Carlos Palacios (Universidad de Salamanca, Spain)

49 **Free-Range Hen Activity with Outdoor Restrictions Measured by Tri-Axial Accelerometers**
José-Alfonso Abecia (Universidad de Zaragoza, Spain); Roberto Vitaller and Marimar Campo (IA2, Departamento de Producción Animal y Ciencia de los Alimentos, Universidad, Spain)

S3.3 - Metrology for Food Quality, Safety, and Traceability - PART III

Room: Sala Ganimede

50 **Enumeration of Enterobacteriaceae in Foodstuffs by BS EN ISO 21528-2:2017: Is It Time to Change It?**
Roberta Tolosi, Evgeniia Igosheva, Riccardo Miotti Scapin and Valerio Giaccone (University of Padua, Italy); Federica Giacometti (University of Padova, Italy)

51 **Quantifying E. Coli in Bivalve Molluscs: A Comparative per-Formance Evaluation of MPN and Pour-Plate Techniques**
Beatrice Testa (University of Naples Federico II, Italy); Rosa Luisa Ambrosio (University Federico II Naples, Italy); Valeria Vuoso (University of Naples, Italy); Giusi Fiore, Raffaele Marrone and Aniello Anastasio (University of Naples Federico II, Italy)

52 **Risk Assessment of Staphylococcus Aureus in Ripened Artisanal Goat Cheeses**
Giusi Fiore (University of Naples Federico II, Italy); Valeria Vuoso (University of Naples, Italy); Beatrice Testa (University of Naples Federico II, Italy); Carmen Cabato (University of Federico II of Naples, Italy); Aniello Anastasio (University of Naples Federico II, Italy); Rosa Luisa Ambrosio (University Federico II Naples, Italy)

53 **Italian Beef is Not a Source of Potentially Toxic Elements for General Population: Updated Evidence**
Maria Olga Varrà and Giovanni Tommaso Lanza (University of Parma, Italy); Lenka Husáková (University of Pardubice, Czech Republic); Federico Ferrari (University of Parma, Italy); Valentina Lorenzi (Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia-Romagna, Italy); Luigi Bertocchi (Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Italy); Martina Piroutkovà and Jan Patočka (University of Pardubice, Czech Republic); Sergio Ghidini (University of Milan, Italy); Emanuela Zanardi (University of Parma, Italy)

54 **Blood-Based Biomarkers of Frailty in Aging Dogs: Preliminary Results from the OLD DOG Longitudinal Study**
Polina Zemko (University of Padua, Italy); Tommaso Banzato (University of Padova, Italy); Federico Bonsembiante (University of Padua, Italy)

55 **Modernizing the Dairy Food Chain: A Data-Driven Innovation for Smart Farming**
Francesca D. Sotgiu (University of Sassari, Italy); Francesco Martini (Abinsula Srl, Italy); Nicoletta Deiana (Abinsula srl, Italy); Tiziana Fanni and Silvia Macis (Abinsula Srl, Italy); Katiuscia Zedda (Abinsula srl, Italy); Matteo Sini, Andrea Mattu, Antonio Spezzigu, Pietro Masala, Alberto Atzori, Antonello Ledda and Giovanna Seddaiu (University of Sassari, Italy); Ciriaco Ligios and Riccardo Bazzardi (Istituto Zooprofilattico Sperimentale della Sardegna, Italy); Fiammetta Berlinguer (University of Sassari, Italy)

S4.1 - Smart sensing and computer vision applications for monitoring animal behaviour, health, and welfare - PART II

Room: Sala Luna

56 **Monitoring Lambing Behavioral Changes in Ewes Using Tri-Axial Accelerometry**
Setayesh Ghadir and Delaram Ghadir (CNIT, Italy); Adele Frau (Università di Sassari, Italy); Tesfalem Mehari Berhe (CNIT, Italy); Francesca D. Sotgiu and Julien Spyros Karel D'Hallewin (University of Sassari, Italy); Davide Adami (CNIT Pisa Research Unit, University of Pisa, Italy); Stefano Giordano (University of Pisa, Italy); Francesca Mossa and Fiammetta Berlinguer (University of Sassari, Italy)

57 **Assessing Welfare in Dairy Cows During Hoof Trimming Through Infrared Thermography**
Gaia Pesenti Rossi, Carolina Giorgiutti, Sara Barbieri, Emanuela Dalla Costa and Elisabetta Canali (Dept. of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy)

58 **A Design of Digital Twin for Smart Dairy Farming: A Systematic Review**
Anisseh Sobhani (University of Padova, Italy); Alessia Zoroaster and Barbara Contiero (University of Padua, Italy); Giulio Cozzi and Paolo Berzaghi (University of Padova, Italy); Ben Aernouts (KU Leuven, Belgium); Fernando Estellés (Universitat Politècnica de València, Spain); Lorenzo Serva (University of Padua, Italy)

59 **Reading Between the Lines: An Algorithm for Electroencephalogram (EEG) Analysis in Horses**
Angela Perrone Barbosa (Universidade de São Paulo, Brazil); Francesco Maria Tagorra (Università degli Studi di Milano, Italy); Tiago Marcelo Oliveira (University of São Paulo, Brazil); Chiara Maccario (Università degli Studi di Milano, Italy); Elie Atallah (Università Degli Studi di Milano, Italy); Raquel Yvonne Arantes Baccarin (Universidade de São Paulo, Brazil); Emanuela Dalla Costa (Università Degli Studi di Milano, Italy)

S4.2 - Advanced Measurement Systems and Data-Driven Solutions for Sustainable and Climate-Resilient Livestock Systems - PART I

Room: Sala Europa

60 **In-Field Validation of an IoT-Based System for Monitoring Ammonia and Fine Particulate Matter from Livestock Buildings**
Provvidenza Rita D'Urso (University of Catania, Italy); Grazia Cinardi (Università di Catania, Di3A, Italy); Luciano Manuel Santoro, Hugo Pennanech and Giovanni Cascone (University of Catania, Italy); Francesco Bonavolontà (Università di Napoli Federico II, Italy); Claudia Arcidiacono (University of Catania, Italy)

61 **Quantifying NH3 Emissions in Naturally Ventilated Dairy Barns: A Decade of Methodological Advancements and Challenges**
Alessandra Apostolico, Ester Scotto di Perta and Elena Cervelli (University of Naples Federico II, Italy); Tomas Norton (KU Leuven - University, Belgium); Stefania Pindozzi (University of Naples Federico II, Italy)

62 **Low-Cost Drone System for Monitoring Ammonia and GHG Emissions in Dairy Barns: A Case Study in Southern Italy**
Valentina Becciolini, Alessio Mattia and Giuseppe Rossi (University of Florence, Italy); Provvidenza Rita D'Urso (University of Catania, Italy); Grazia Cinardi (Università di Catania, Di3A, Italy); Claudia Arcidiacono (University of Catania, Italy); Leonardo Conti (University of Florence, Italy)

63 **High-Frequency Environmental Measurements for Data-Driven Livestock Management**
Ambra Milani and Francesco Corradini (Cynomys, Italy)

64 **An IoT-Based Multi-Gas Monitoring Platform for Real-Time Air Quality Assessment in Livestock Buildings**
Elia Landi, David Baldo, Stefano Parrino, Marco Tani, Ada Fort, Marco Mugnaini, Irene Cappelli and Enza Panzardi (University of Siena, Italy)

S4.3 - Precision Measurement for Sustainable and Welfare-Oriented Pig Farming Systems

Room: Sala Ganimede

65 **Evaluation of an Automatic Feeding System: Effects on Lactating Sow Feed Intake and Piglet Growth**
Daniele Favaro (University of Padua, Italy & Freelance Veterinary Doctor - SUIVET, Italy); Matilde Campara, Flaviana Gottardo and Barbara Contiero (University of Padua, Italy); Elisa Moscon (Freelance Veterinary Doctor - SUIVET, Italy); Francesco Tonon (University of Padua, Italy & Freelance Veterinary Doctor - SUIVET, Italy)

66 **Precision Livestock Farming for Tail Biting Management in a Commercial Breeding Environment**
Guido Di Martino (Experimental Zooprophylactic Institute of the Veneto, Italy); Aurora Ambrosi, Vittoria Tregnaghi, Rachele Urbani, Giacomo Nalesso and Massimiano Bassan (Istituto Zooprofilattico Sperimentale delle Venezie, Italy); Valentina Bonfatti (University of Padua, Italy); Giovanni Alborali (Istituto Zooprofilattico Sperimentale della Lombardia e dell Emilia Romagna, Italy); Giorgio Provolo (University of Milan, Italy); Grazia Manca (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)

67 **Economic Impact of Porcine Epidemic Diarrhea Virus in Growing Pigs: An Integrated Algorithm Combining Causal Inference Analysis and a Full-Budget Model**
Alice Perrucci (University of Turin, Italy & Struttura Srl, Manerbio, BS, Italy); Heitor De Avila (Dept. of Animal Science, Iowa State University, US, Brazil); Annalisa Scollo (University of Torino, Italy); Edison Magalhaes (Dept. of Animal Science, Iowa State University, US, USA)

68 **Piglet Crushing Mechanisms in Temporary Crating Systems: Insights from Continuous Video Monitoring**
Maria Costanza Galli and Letizia Leone (University of Turin, Italy); Annalisa Scollo (University of Torino, Italy)

Thursday, April 30

S5.1 - Biosensors and AI for Diagnostics in Veterinary and Animal Sciences

Room: Sala Luna

69 **Unmasking the Underdog: Advanced Molecular Strategies to Improve Piroplasm Characterisation and Unveil Co-Infections in Dogs and Ticks**
Marika Grillini (University of Padua, Italy); Hana Tadesse (Addis Ababa University, Ethiopia); Tingxi Zhang (University of Padua, Italy); Naledi Sekgobela (University of Pretoria, South Africa); Giorgia Dotto and Giovanni Franzo (University of Padova, Italy); Bersissa Kumsa (Addis Ababa University, Ethiopia); Raksha Bhoora (University of Pretoria, South Africa); G. Simonato (Key Laboratory of Livestock Infectious Diseases in Northeast China, Ministry of Education, College of Animal Science and Veterinary Medicine, Shenyang Agricultural University, Shenhe District, Shenyang, No.120, Dongling Road, 110866, China); Rudi Cassini (Unievrsity of Padova, Italy)

70 **Molecular Detection and Genospecies Diversity of Borrelia in Ixodes Ricinus Ticks from an Endemic Area of Northeastern Italy**
Filippo Marzoli, Federica Obber, Alessia Franzoso, Erica Maria Fabiano, Carlo Vittorio Citterio, Ilenia Drigo and Graziana Da Rold (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)

71 **Molecular Detection of Echinococcus Multilocularis and Cestode Infections in Alpine Small Rodents**
Graziana Da Rold, Filippo Marzoli, Alessia Franzoso, Erica Maria Fabiano, Carlo Vittorio Citterio and Federica Obber (Istituto Zooprofilattico Sperimentale delle Venezie, Italy); Rudi Cassini (Unievrsity of Padova, Italy); Erica Marchiori and Leonardo Turioni (University of Padua, Italy); Eva Ladurner (Museum of Nature South Tyrol, Italy)

72 **Integrating Lactation and Dry off Data to Predict Post Calving Intramammary Infections in Dairy Cows**
Luca Barin, Paolo Balasso and Giorgio Marchesini (University of Padua, Italy); Antonio Barberio (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)

73 **Detection of Coronet Temperature Changes in Horses Using Infrared Thermography During Partial Limb Occlusion and Reperfusion**

Chiara Maccario (Università degli Studi di Milano, Italy); Jil Jessy Kellershohn (Vetsuisse-Fakultät Universität, Swaziland); Annika Bremhorst (Vetsuisse-Fakultät Universität, Switzerland); Livia Dumont (Veterinary Dept. of the Swiss Armed Forces); Stéphane Montavon (Swiss Armed Forces, Switzerland); Claudia Spadavecchia (Vetsuisse-Fakultät Universität, Switzerland); Feighelstein Marcelo, Sr. (Engineering Cluster, Tel-Hai University of Kiryat Shmona on the Galilee, Israel); Anna Zamansky (University of Haifa, Israel); Emanuela Dalla Costa (Università Degli Studi di Milano, Italy)

74 **Sequence-Informed Machine Learning to Predict Antigenic Distance Among Newcastle Disease Virus (NDV) Strains**

Giovanni Franzo (University of Padova, Italy); Alice Fusaro and Isabella Monne (Istituto Zooprofilattico Sperimentale delle Venezie, Italy); Mattia Cecchinato (Padua University, Italy); Alessio Bortolami (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)

S5.2 - Advanced Measurement Systems and Data-Driven Solutions for Sustainable and Climate-Resilient Livestock Systems - PART II

Room: Sala Europa

75 **Subclinical Ketosis as a Driver of Enteric Greenhouse Gas Emissions in Dairy Cows**

Giorgia Taio (University of Padua, Italy); Lisuzzo Anastasia (University of Padua, Legnaro (PD), Italy); Erminio Trevisi (Università Cattolica del Sacro Cuore Piacenza, Italy); Fiorenzo Piccioli-Cappelli (Università Cattolica del Sacro Cuore, Piacenza (PC), Italy); Francesca Arfuso and Elisabetta Giudice (University of Messina (ME), Italy); Federico Froldi (Università Cattolica del Sacro Cuore, Piacenza (PC), Italy); Francesca Cecchini and Matteo Gianesella (University of Padua (PD), Italy); Enrico Fiore (University of Padua, Legnaro (PD), Italy); Michele Berlanda (University of Padua (PD), Italy)

76 **Comparison Among Different Variables from a Laser Methane Detector (LMD) in a Feeding Trial with Fattening Bulls**

Fabio Palmiro Abeni (Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Italy); Stefania Barzaghi (Council for Agricultural Research and Economics (CREA), Italy); Lucia Zingale (CREA, Italy); Andrea Scarabello (Associazione Produttori Carni Bovine, Legnaro (PD), Italy); Luciano Migliorati (CREA, Italy)

77 **Measurement Systems and Policy-Oriented Software Tools for CH4, CO2 and NH3 Emissions in Livestock Systems**

Giulia Pastorelli (CREA, Italy); Giacomo Contò (CREA Research Centre for Animal Production and Aquaculture, Italy); Claudia Ciarelli (ISMEA - the Institute for Services to the Agricultural and Food Market, Italy); Ilaria Falconi (CREA Research Centre for Agricultural Policies and Bioeconomy, Italy)

79 **Assessment of Air Quality and Emissions in Naturally Ventilated Pig Houses Using an IoT Gas and Environmental Sensors Device**

Alberto Finzi (Università Degli Studi di Milano, Italy); Antonio Mautone (Università degli Studi di Milano, Italy)

S5.3 - Precision Nutrition Technologies in Animal Husbandry: Advancements and Future Perspectives

Room: Sala Ganimede

80 **Morphometric Variability of Cervical and Thoracic Circumferences in Holstein Dairy Cows Across Lactation Stage and Age**

Martina Lamanna, Riccardo Colleluori, Marco Canova, Annamaria Grandis, Claudia Giannone and Marco Bovo (University of Bologna, Italy); Francesco Petretto (IP Ingegneria SRL, Italy); Andrea Formigoni and Damiano Cavallini (University of Bologna, Italy)

81 **Blood Metabolomic Characteristics in Dairy Cows Treated with Hepato-Protectors During the Transition Period**

Lisuzzo Anastasia (University of Padua, Legnaro (PD), Italy); Alessio Valenza (Ceva Animal Health, Italy); Luca Laghi (University of Bologna, Italy); Giorgia Taio (University of Padua, Italy); Matteo Gianesella (University of Padua (PD), Italy); Andrea Biancucci (Italy); Enrico Fiore (University of Padua, Legnaro (PD), Italy)

82 **Cyromazine Contaminated Substrate: Effects on Life-History Characters and Heat Resistance of Hermetia Illucens**
Asia Zanzot (University of Pisa, Italy); Tara N. McNeil and Jeffery K. Tomberlin (Texas A&M University, USA); Simone Mancini (University of Pisa, Italy)

83 **Antiproliferative Action of Diketopiperazines Extracted from Vinery by-Products in a Sheep Model**
Maria Giovanna Ciliberti, Marzia Albenzio, Matteo Francavilla, Cristina Stango, Antonella Santillo, Mariangela Caroprese and Agostino Sevi (University of Foggia, Italy)

84 **Assessing Liveweight Dynamics and Production Responses to Nutritional Level in Sarda Dairy Ewes Through Walk-over-Weighing Technology**
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85 **GPS-Based Assessment of Pasture Space Use in Nera di Arbus Sheep to Support Grazing Management**
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Ignacio Contreras Solís (Universidad Pública de Navarra, Spain)

Sustainability assessment of small ruminant farming system in Northern Apulia using the FAO SAFA framework

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In the Apulia region, sheep and goat farming represents an important agricultural activity, characterized by marked heterogeneity, encompassing farms that differ in size, production aims, breeds, and levels of intensification, which may exert distinct impacts on environmental and socioeconomic conditions [1]. During the last years, different tools and methods have been developed to assess different aspects of the sustainability at the farm level. Although these tools provide valuable insights, they often focus on environmental impacts or economic efficiency and may insufficiently address social and governance-related aspects. The Food and Agriculture Organization of the United Nations (FAO) developed the Sustainability Assessment of Food and Agriculture Systems (SAFA), an integrative approach for the development of a comprehensive sustainability framework [2]. Therefore, the aim of this study was to assess the sustainability performance of small ruminant farming systems in Northern Apulia (Italy) using the SAFA framework, to identify strengths, weaknesses, and priority areas for improvement. Ten small ruminant farms, representative of the study area, were selected through a stratified sampling approach based on structural characteristics of livestock farms, considering also farm size, production orientation, and management characteristics. Data were collected through structured face-to-face interviews using an ad hoc questionnaire developed in accordance with the SAFA guidelines comprising 110 questions and scored using the five-point rating system across Good Governance (13 indicators), Environmental Integrity (52 indicators), Economic Resilience (26 indicators), and Social Well-being (19 indicators) dimensions. From the primary data collected, a dataset was built to run a descriptive analysis. Good Governance data revealed a polarised governance profile, with very strong performance in formal commitments and management systems, but significant weaknesses in implementation, accountability, and stakeholder participation emerged. Environmental Integrity demonstrated a highly uneven performance, with strong results in local pollution control, water quality, soil management, and animal welfare, but contrasted by substantial weaknesses in climate mitigation, biodiversity conservation, and resource efficiency. The Economic Resilience dimension displayed high scores in financial management, supply-chain stability, and product quality, alongside notable weaknesses in supplier dependence, risk-sharing mechanisms, and certain food safety practices. Social Well-Being data evidenced high scores for indicators related to formal rights, non-discrimination, health and safety, and cultural recognition, contrasted by critical weaknesses in material living conditions, fair economic relations, and remuneration. Overall, the findings highlight the resilience and socio-cultural value of traditional small ruminant farming systems, while also identifying opportunities for targeted interventions, such as strengthening governance structures, improving management practices, and promoting economic diversification, to enhance sustainability across all the SAFA dimensions.

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Advancing nutritional life cycle assessment (nLCA): Evaluating functional units for integrating nutrition, health, and environmental impact of animal sourced foods

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Nutritional life cycle assessment (nLCA) extends traditional life cycle assessment (LCA) by integrating environmental impacts with nutritional and health outcomes [1]. Unlike mass-based LCA functional units (e.g., kg CO_2e/kg product), nLCA employs nutritionally informed functional units to contextualize environmental footprints in terms of human health relevance [2]. Despite growing interest, standardized and quality-aware nutritional functional units remain underdeveloped (Figure 1).

This study systematically identifies, compares, and critically evaluates nutritional functional units of animal-sourced foods within nLCA, emphasizing the incorporation of nutritional quality metrics to inform sustainable dietary guidance. Medline, Scopus, and Web of Science were systematically searched using rigorous inclusion and exclusion criteria. Screening and data extraction were independently performed by two reviewers, with discrepancies resolved by a third. Data extraction included environmental impacts, public health indicators, and nutritional functional units.

Twenty seven studies were included in the systematic review. Identified functional units included protein content, protein quality metrics (e.g., Digestible Indispensable Amino Acid Scores, DIAAS), omega-3 fatty acids, and Nutrient Density Scores (NDS). While many studies quantified nutritional function, few integrated nutrient quality parameters such as bioavailability, amino acid balance, or digestibility, highlighting a critical gap in current nLCA methodologies.

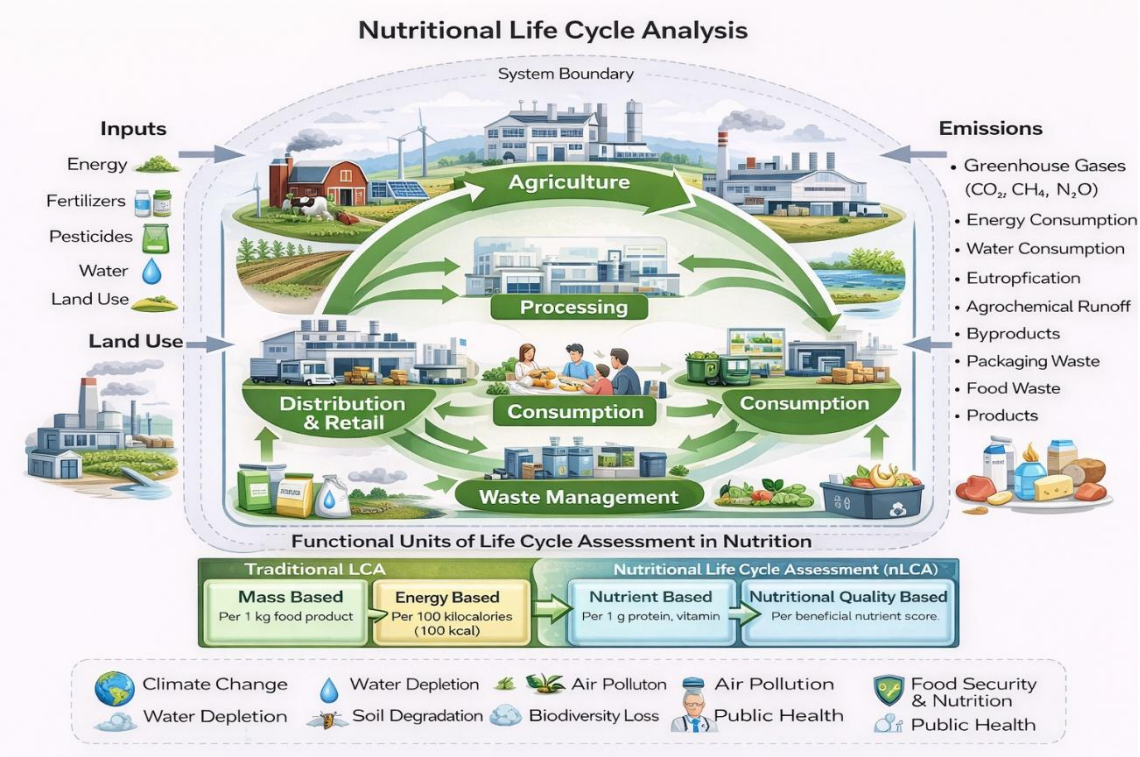


Figure 1: Conceptual Framework of Nutritional Life Cycle Assessment (nLCA) Across the Food System (created using ChatGPT)

Novelty and Future Direction: This work identifies the synthesis of nutritional functional units with quality-adjusted metrics, providing a framework for assessing the true environmental and nutritional value of animal-sourced foods. Such analyses could inform a wide range of applications across food system stakeholders. Policymakers may leverage this approach to develop national healthy and sustainable dietary guidelines, tailor institutional meal planning and nutrition campaigns, redirect agricultural subsidies towards nutrient-dense and low-impact food groups, establish taxation frameworks, and strategically plan agricultural activities. Food manufacturers could evaluate the positioning of their product portfolios against nutritional and environmental metrics, guiding restructuring or diversification decisions. Programme managers across governmental, non-governmental, and private sectors could use these insights to prioritize context-specific interventions targeting impactful food groups.

Looking forward, dynamic nLCA platforms integrating multi-nutrient quality indices, predictive environmental modelling, and personalized dietary guidance could revolutionize sustainable food systems. By bridging nutrition, health, and environmental outcomes, nLCA can unlock decision-making tools for policymakers, industry, and programmatic actors, creating opportunities for innovation and transformation in diets and food production.

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Direct prediction of beef eating quality from raw muscle using REIMS spectral output

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Rapid Evaporative Ionisation Mass Spectrometry REIMS is an ambient analytical technology that enables real time chemical fingerprinting of biological materials. While REIMS has been applied to tissue identification, food authentication and selected meat quality traits [1, 2], its ability to directly relate raw muscle chemistry to consumer sensory scoring not fully understood or researched

This study aimed to integrate REIMS with advanced chemometric modelling to connect raw beef muscle profiles with maturation time and consumer sensory scoring within a single analytical framework. A total of 149 beef samples were collected from 31 carcasses representing four muscles. Carcasses were processed using two hang methods and sampled following 7 and 21 days of maturation post slaughter. Consumer taste panels were conducted across 18 locations to generate sensory scores.

Samples were analysed using a monopolar electrosurgical iKnife coupled to a Waters REIMS source and a Xevo G2 XS QTof mass spectrometer operating in negative ionisation mode. Spectral data were processed using Abstract Model Builder and multivariate statistical analysis was conducted in Simca to develop classification and discrimination models.

REIMS successfully discriminated between the four beef muscles and differentiated samples matured for 7 and 21 days (Figure 1). Importantly, chemical fingerprints derived from raw muscle were also positively discriminative for quality traits determined by consumer sensory scoring. These findings demonstrate that molecular patterns captured at the point of sampling reflect attributes that are directly relevant to eating quality as perceived by consumers.

The uniqueness of this work lies in the direct linkage of REIMS data from raw beef to consumer sensory scoring outcomes using a unified chemometric approach. This represents a significant step towards objective prediction of eating quality from intrinsic muscle chemistry without reliance on time intensive post processing or destructive laboratory assays. The integration of REIMS with multivariate modelling provides a foundation for rapid classification tools capable of supporting quality assurance, value-based marketing and more precise supply chain decisions in the beef sector.

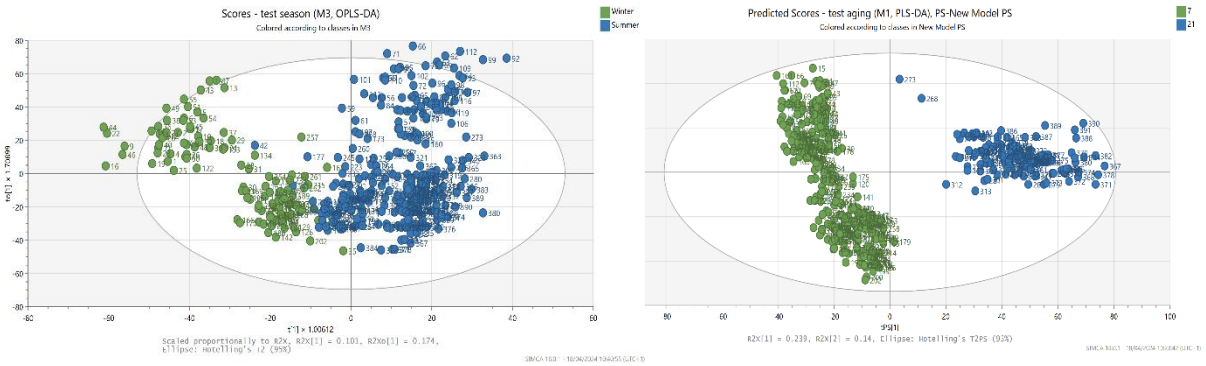


Figure 1: OPLS-DA analysis for winter and summer finishing (left) 7- and 21-days maturation (right)

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How do conventional, organic and free-range pig farming systems differ in their environmental impacts? A meta-analysis and multi-variate analysis of pig life cycle assessment studies

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Pig production is a major component of global livestock supply chain. At the same time, it contributes substantially to agricultural greenhouse gas emissions (GHG) and other environmental burdens, primarily through feed production, manure management, land occupation, and on-farm energy use [1]. Life cycle assessment (LCA) is the methodological framework used to quantify the environmental impacts of pig production. However, available LCA outcomes remain highly heterogeneous due to variations in system boundaries, allocation procedures, emission factors, regional contexts, feeding strategies and production systems [2-3]. These constrain the derivation of robust conclusions regarding the environmental performance of conventional and alternative pig farming systems. The present study addresses this gap through a comprehensive systematic review and meta-analysis of pig LCA studies, with particular emphasis on comparing pig farming systems, i.e., conventional, organic, and free-range. Beyond quantifying differences, the ultimate objective is to identify structural patterns in environmental performance and derive thresholds that can be used in multicriteria analyses within the 'One Quality' framework [4].

Thus, we conducted a systematic literature review following the STARR-LCA protocol and PRISMA reporting guidelines [2]. A total of 93 attributional pig LCA studies were included. Analyses presented here focus on studies using kg live weight (LW) only as a functional unit. Therefore, 75 studies were used yielding 266 individual treatments (Figure 1). Five major environmental impact categories were consistently reported in pig LCA studies. These are projected in the principal component analysis (PCA, Figure 1) along of key animal performance and production-stage characteristics. A clear system-level structuring is revealed. Free-range system was distinctly separated and exhibited a large dispersion, indicating substantial heterogeneity in environmental profiles. This reflects variability in feed sourcing, stocking density, growth performance, and land occupation patterns characteristic of outdoor systems. Organic farming has lower within-group variability but systematic divergence from conventional farming along axes associated particularly with land use and energy demand. Conventional farming reflects the dominant system (>89%). The PCA further indicated that CC and NREU were well aligned, confirming the dominant contribution of feed production and energy inputs to overall GHG. The projection of LU emphasizes the distinct influence of production extensification, mainly in organic and free-range systems. These data demonstrate that differences among pig farming systems are not merely attributable to magnitude variations in single impact categories but reflect distinct configurations of emission drivers. The meta-analysis enabled also to derivate percentile-based threshold ranges for each impact category within and across systems. The results underscore the importance of accounting for production modality when interpreting LCA outcomes and demonstrate that trade-offs across impact categories are intrinsic to farming system design.

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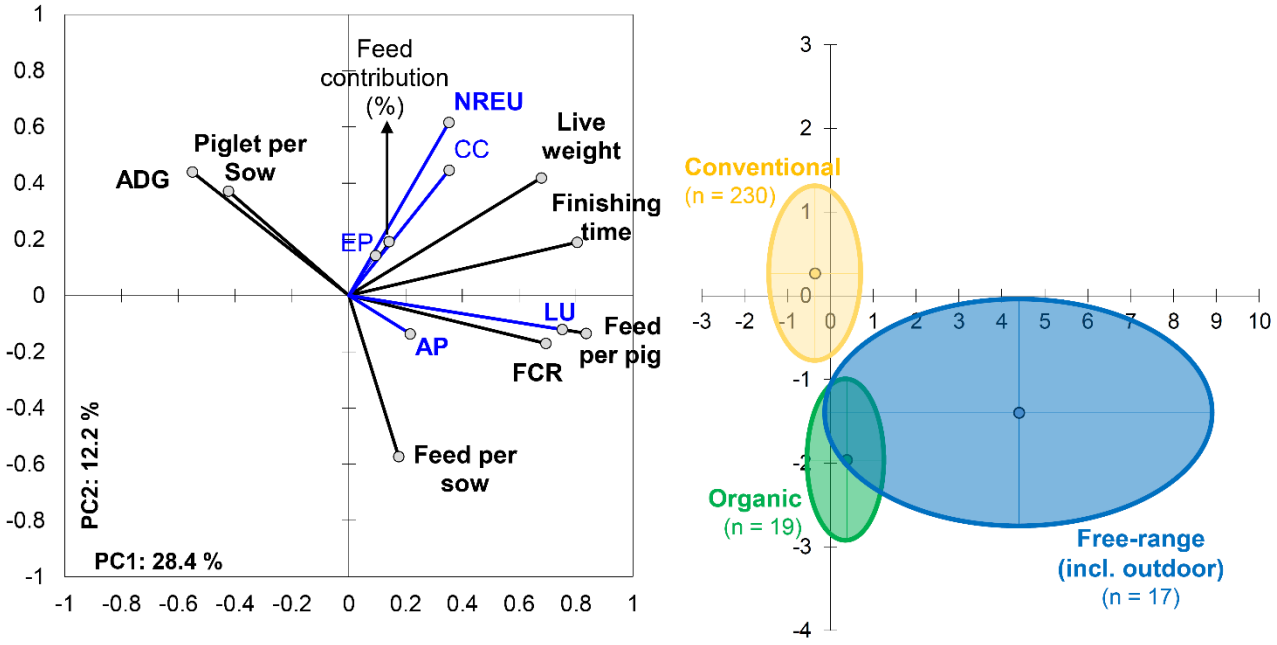


Figure 1 – PCA of environmental impacts and associated animal performance indicators within 3 pig farming systems. Blue vectors are the environmental impact categories: CC (climate change, kg CO_2-eq), LU (land use, $m^2 \cdot$year), AP (acidification potential, g SO_2-eq), EP (eutrophication potential, g PO_4^{3-}-eq), and NREU (non-renewable energy use, MJ). Black vectors are animal performance and production-stage characteristics, including ADG (average daily gain, g/day) and FCR (feed conversion ratio). The variables that are significantly different within the three farming systems are in bold. Ellipses indicate the distribution of treatments within the 3 farming systems (total data points for each system is given).

Development and validation of portable NIRS calibration models for predicting fecal composition

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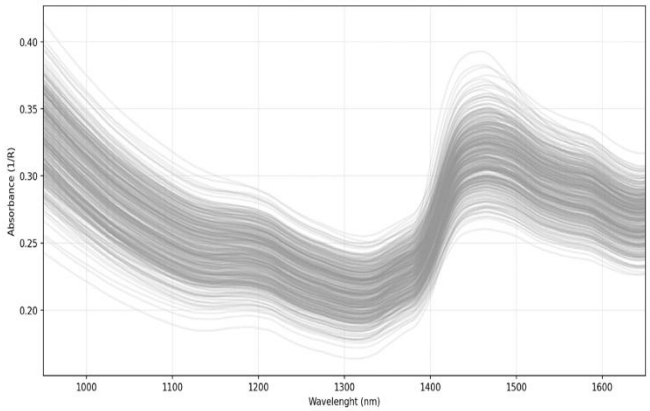


Figure 1 - Raw NIR spectra (950-1650nm) acquired with Aurora NIR.

The fecal analysis to monitor ruminant nutrition offers valuable insights into digestive efficiency and dietary adequacy, but conventional laboratory methods remain costly and impractical for routine application [1]. Near-infrared spectroscopy (NIRS) represents a rapid and non-destructive alternative; however, its application to buffalo feces, especially using portable devices, remains largely unexplored. The present study aimed to develop and validate calibration models for a portable NIRS device for predicting the chemical composition of buffalo feces in terms of dry matter (DM), crude protein (CP), ether extract (EE), ash, fiber fractions ($aNDF_{om}$, ADF, ADL) and starch. A total of 326 fecal samples were collected from lactating buffaloes and analyzed using conventional reference methods [2]. The samples were pre-dried at 65 °C until constant weight and ground (1mm). Subsequently, each sample was placed in a round cell and scanned in quadruplicate in reflectance mode using a portable NIRS device (AuroraNIR, GraiNit S.R.L., Italy) with a wavelength operative range of 950-1650nm (Fig.1); the calibration models were developed using different spectral pretreatments and multivariate regression techniques available on UCall^{TM}software (Unity-Scientific, Australia), furnished with AuroraNIR.

The dataset was randomly split into 70% for calibration and 30% for validation; models'performance was assessed through internal cross-validation (k-fold method) and independent validation, using coefficient of determination (R^2), standard error of calibration (SEC) and validation (SEV), residual predictive deviation (RPD) and Akaike information criterion (AIC, Δi) [3]. Results highlighted differences among constituents. Ash and CP showed the most acceptable predictive performance, achieving moderate $R^2$and acceptable error metrics in both calibration (R^2_C=0.74, RPD=1.61, Δi=0 for Ash and R^2_C=0.81, RPD=1.64, Δi=0 for CP) and validation (R^2_V=0.63, SEV=1.03 for Ash; R^2_V=0.60, SEV=1.01 for CP). Fiber fractions ADF and ADL showed intermediate ability (R^2_C=0.68, RPD=1.60 for ADF; R^2_C=0.67, RPD=1.38 for ADL), whereas DM and $aNDF_{om}$ (R^2_C=0.55, RPD=1.26 for DM; R^2_C=0.56, RPD=1.10 for $aNDF_{om}$) showed limited performance. EE and starch predictions were unsatisfactory due to the worst results for all tested metrics. Although predictive performance was generally lower than that reported in studies using benchtop NIRS [4,5], several constituents achieved comparable results such as for ash, CP, DM and starch [1], despite the narrower spectral range and reduced sensitivity of the portable device. Portable NIRS may therefore represent a useful tool for screening faecal chemical traits in buffaloes, supporting precision feeding while reducing analytical costs and time; however, expanded spectral database and further calibration refinements are needed to reach higher performance standards of such NIRS application.

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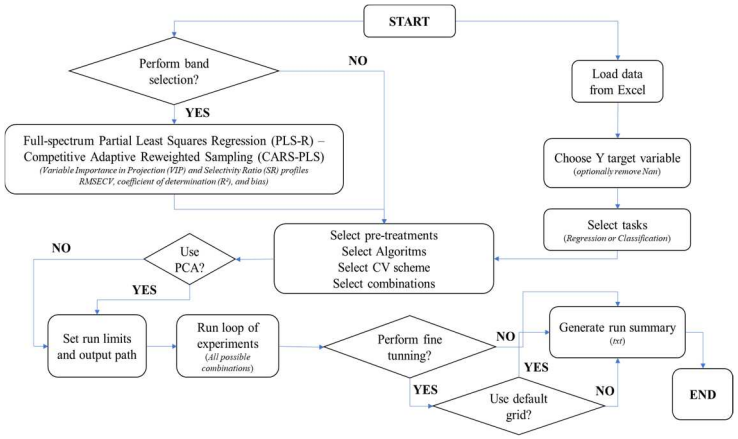
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From Benchtop to Portable Sensors: NIR Spectroscopy and Colour Analysis for Honey Quality and Origin Evaluation

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The assessment of honey quality and authenticity represents a relevant challenge for the agri-food sector, with implications for food safety, consumer protection, and market transparency [1-2]. Near-infrared spectroscopy (NIR), combined with chemometric analysis, has emerged as a rapid and non-destructive analytical approach; however, the comparative performance of benchtop and portable NIR instruments and the robustness of associated modelling workflows require systematic evaluation [2-5].

In this study, eighty Italian honey samples from different botanical origins were analysed using a benchtop NIR spectrometer (FOSS DS-2500, FOSS Analytical A/S, Hillerød, Denmark) and two portable NIR devices (NeoSpectra™, Si-Ware Systems, Menlo Park, CA, USA; and AlbaNIT, GraiNit s.r.l., Padua, Italy). Colourimetric measurements were performed using a Konica Minolta CD-600 colorimeter (Konica Minolta Sensing Inc., Japan).

Chemical parameters, including moisture, electrical conductivity, sugars, pH, diastatic activity, and hydroxymethylfurfural content, were used as reference data for model calibrations and validations. Spectral data were processed through a comprehensive chemometric workflow (**Figure 1**: *Schematic workflow adopted for data analysis*) involving spectral pre-processing, wavelength selection (siPLS and CARS-PLS), and multivariate and machine learning algorithms for both regression and classification tasks. Model performance was evaluated using Venetian-blind cross-validation and bootstrap resampling.

The benchtop instrument achieved the highest predictive accuracy, with coefficients of determination (R^2) up to 0.91 for electrical conductivity and glucose content, and high performance in floral origin classification (balanced accuracy up to 0.90). Portable devices showed variable but promising results, particularly for selected quality parameters such as glucose, pH, and electrical conductivity, although classification performance remained moderate. Spectral band selection improved model parsimony and, in some cases, predictive accuracy, highlighting the relevance of wavelength regions associated with water and carbohydrate absorption.

These results demonstrate the potential of NIR spectroscopy combined with tailored chemometric workflows for honey quality assessment. While benchtop instruments currently offer superior analytical performance, portable NIR devices represent a viable option for rapid screening and on-field applications, supporting the integration of sensor-based measurements into routine quality control systems.

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Critical Importance of pH and a_w Measurement Site in Dry-Aged Meat and Its Implications for Food Safety

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Adequate sampling for collection of data that characterize food products are critical in assessing their safety. This is underlined within Regulation (EC) No 2073/2005 [1] explicitly regarding pH and water activity (a_w). The choice of the sampling site is not only essential for the correct execution and interpretation of challenge tests, but also becomes increasingly relevant in view of the growing reliance on predictive microbiology as a rapid and cost-effective tool for food safety management.
In dry-aged meat (Reg 2024/1141) [2], marked variability of pH and a_w occur on the surface as a consequence of dehydration, proteolysis, and environmental exposure. Throughout the process, food business operators may apply parameters combinations, namely temperature relative humidity and airflow other than the ones set by regulation, if they demonstrate to the satisfaction of the competent authority that equivalent guarantees are provided on the safety of the meat.
The aim of the present study was to evaluate whether sampling sites affect the pH and a_w values and, consequently, the outcomes of predictive models.
For the trial, beef eye of round samples were collected in triplicate (3 independent batches) at each time point during a 45-day aging period: prior to the onset of dry-aging (T0) and after 15 (T15), 30 (T30), and 45 (T45) days, from both superficial (5 mm) and inner (20 mm) portions. At each time point and for each batch, all measurements were performed in triplicate. With regard to pH, a constant value was observed on the surface, ranging from 5.40 (± 0.04) to 5.37 (± 0.05) at T0 and T45 respectively. In contrast, at greater depth a steady (Δ= 0.35) increase in pH was detected from T0 to T45, ranging from 5.60 to 5.95. For a_w the surface portion exhibited a marked decrease (Δ= 0.07) from 0.992 (± 0.003) at T0 to 0.919 (± 0.001) at T45, reflecting the pronounced dehydration of the crust, whereas at greater depth the loss was lower but still evident (Δ= 0.02), from 0.992 (± 0.002) at T0 to 0.960 (± 0.004) at T45.
Different scenarios emerge when applying the ComBase predictive model using data collected from either the superficial or the inner part of the product with a temperature reproducing the internal condition of the aging chamber. Specifically, *Listeria monocytogenes* growth predictions already differ from T15 showing a high overestimation if compared with challenge test, when inserting inner data. Besides, lower values are foreseen when using the data from the superficial area, but still not comparable to real scenarios.
Inappropriate sampling strategies may generate biased datasets, inevitably leading to inaccurate or misleading predictions. Consequently, a scientifically sound, risk-oriented definition of the sampling point is a prerequisite to ensure regulatory compliance and the robustness of predictive microbiology applications to dry-aged meat products.

[1] Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

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[2] Commission Delegated Regulation (EU) 2024/1141 of 14 December 2023 amending Annexes II and III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards specific hygiene requirements for certain meat, fishery products, dairy products and eggs

Virulence Potential and Antimicrobial Resistance of *Bacillus cereus* Isolated from Infant Formulae

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Powdered infant formulae may be contaminated by pathogenic microorganisms. Spore-forming bacteria can survive production and shelf life [1]. *Bacillus* spp. is frequently isolated [2], and *Bacillus cereus* can compromise product quality and safety through spoilage, toxin production or infection [3].

This study aimed to characterize pathogenic *B. cereus* strains isolated from powdered formulae. During the microbiological evaluation of 83 formulae (23 infant formulae, 42 follow-on formulae, and 18 formulae for special medical purposes), 42 *B. cereus* strains were isolated. Three strains were recovered by direct enumeration on PEMBA agar, while 39 were isolated after enrichment in buffered peptone water followed by streaking onto PEMBA agar.

For phenotypic characterization, hemolysin BL production was assessed by measuring hemolysis zones on Columbia blood agar supplemented with 5% sheep blood (after incubation at 30°C for 18 h). Phosphatidylcholine-specific phospholipase C (PC-PLC) activity was evaluated by measuring precipitation halos on Blood Agar Base supplemented with 0.15% L-α-phosphatidylcholine. Proteolytic activity was assessed by measuring clear zones around colonies on 1.2% agar plates containing 1.5% skim milk (37°C for 18 h).

For toxin-related genes, strains were grown in liquid culture, DNA was extracted and purified. Conventional PCR was performed targeting genes encoding sphingomyelinase (sph), enterotoxins (entFM, entS, bceT), non-hemolytic enterotoxin components (nheA, nheB, nheC), cytotoxin K (cytK), and cereulide (ces). Antimicrobial susceptibility of the 42 strains was assessed by broth microdilution using the Sensititre™ automated system with the GPALL1F plate. Then, minimum inhibitory concentrations were interpreted according to EUCAST 2025 criteria.

Only two strains harboured all virulence factors except ces or cytK. All strains exhibited proteolytic activity, 50% showed PC-PLC activity, and 67% produced hemolysin BL. The prevalence of toxin-encoding genes was as follows: sph (97.7%), entFM (64.3%), entS (66.7%), bceT (45.2%), nheA/B/C (76.2%), cytK (7.1%), and ces (28.3%). Although the prevalence of ces was relatively low, cereulide is of particular concern due to its heat stability and pre-formed nature in food.

Antimicrobial resistance profiles revealed high resistance rates to β-lactams and moderate resistance to erythromycin (26.2%). Overall, 69% of the strains were fully susceptible. All resistant isolates showed resistance to only one antibiotic class, with no multidrug-resistant phenotypes detected.

In conclusion, the frequent detection of virulent *B. cereus* in powdered formula highlights safety concerns, emphasizing the need for continuous monitoring, integration of virulence profiling into risk assessment, targeted surveillance of toxin-producing strains, strict hygiene and handling practices during and after reconstitution to protect vulnerable consumers.

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Dairy cows affected by intramammary infection, subclinical and clinical mastitis: changes in milk metabolome profiles

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Immune and inflammatory responses during mammary tissue infection of dairy cows lead to an increase in SCC in milk. Metabolomics is an important branch of system biology that studies metabolites to elucidate disease etiology and characteristics, to monitor disease and develop biomarkers. Several studies were conducted to study milk metabolomic profiles. However, knowledge about the influence of only intramammary infection on milk metabolome are still lacking. The aim of this study was to investigated the quarter-milk metabolomic profile of dairy cows affected by intramammary infection, subclinical mastitis, and clinical mastitis.

Multiparous Holstein-Friesian dairy cows from one farm. Animals were between 30 and 100 days in milk, not under antimicrobial treatment, and without an history of treatment in the previous 6 months. Among them 80 quarters were enrolled and equally divided in four groups: healthy (H) quarters with SCC<200.000 cells/mL and negative bacteriological milk cultures (BMC); intramammary infection (IMI) with SCC<200.000 cells/mL and positive BMC; subclinical mastitis (SCM) with SCC<200.000 cells/mL, positive BMC, and absence of clinical signs; and clinical mastitis (CM) with SCC<200.000 cells/mL, positive BMC, and presence of clinical signs. Milk metabolomics analysis were conducted by 1H-NMR. Differences in metabolites were assessed with a linear mixed-effects model. A p-value<0.05 was accepted, whereas a 0.05≤p-value≤0.10 was considered as trend to significance.

The SCC were 49.200, 66.100, 1.982.800, and 11.834.700 cells/mL for H, IMI, SCM, and CM, respectively. Main pathogens were Staphylococcus aureus (24.4%) and Escherichia coli (23.3%). 45 metabolites were identified of which 34 differed significantly among groups. 12 metabolites differed only in CM compared to all other groups with greater levels in leucine, taurine, valine, acetate, formate, pyruvate, 5-dodecenoic acid, and 3-hydroxybutyrate, and lower levels in arabinose, galactose, ribose, and ascorbate. Other 18 metabolites changed from H to CM, with a progressive increase of 2-aminoadipate, alanine, dimethylamine, n-acetyl-glycine, and lactate, and decrease of creatine-phosphate, o-phosphocholine, glucose, lactose, maltose, cis-aconitate, carnitine, fumarate, phenylacetate, 2-ketobutyrate, acetoacetate, citicoline, and orotate. In conclusion, both SCM and CM showed impaired energy and lipid metabolisms, and altered systemic energy status with worse conditions during CM. Moreover, milk metabolome suggested a progressive increase in inflammation, blood-milk barrier damage, oxidative stress, and microorganism metabolism. Increased cell proliferation was suspected during IMI and SCM, with progressive substrate consumption in CM.

An Integrated Non-Lethal Deterrence Solution Approach to Mitigate Wolf-Livestock Conflicts

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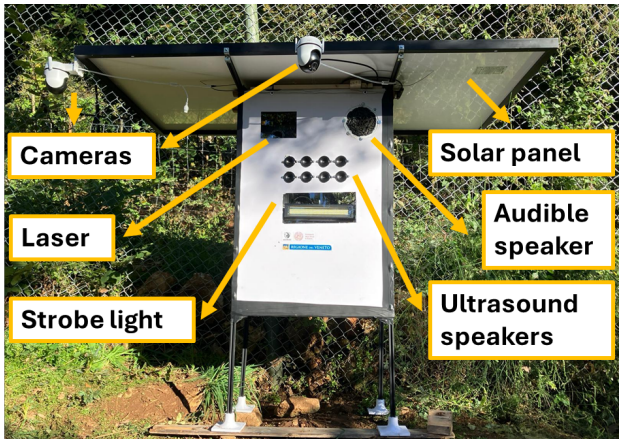


Figure 1: Architecture of the autonomous deterrence system installed in field conditions, showing cameras, actuators (i.e., laser, strobe, audible and ultrasonic speakers), and solar panel.

In the last decades, wolf populations across Europe have increased considerably, expanding into regions where they had been absent before, yet this recovery has also intensified human-wildlife conflict in rural areas.

As the number of wolves increases, the number of livestock predations due to such animals increases too, thus creating significant challenges for farmers and breeders. This is the case of Veneto Region, where traditional grazing activities make livestock especially vulnerable. For instance, in the period ranging from 2015 to 2019, a total of 1157 attacks to livestock were recorded [1]. The most significant challenge is both economic and cultural: farmers and breeders, faced with constant livestock losses, are forced to quit their activities [2]. To mitigate these damages, various deterrence techniques have been implemented.

However, the conventional strategies, such as fladry [3] or culling [4], proven to be largely ineffective, mainly due to wolves' ability to habituate to repeated stimuli, or to ethical concerns related to wolf culling [5]. In this context, the proposed solution (see Figure 1) emerges as a non-lethal alternative featuring a dual deterrence system employing both optical, such as laser and flashing lights, and acoustic stimuli, like audible and ultrasonic (beyond human hearing range but perceptible for wolves). The system is powered by an energy management system equipped with a solar panel, ensuring extended autonomy even in harsh deployment scenarios. Integrated cameras enable automatic wolf detection, triggering the deterrence mechanisms. In addition, thanks to a wireless communication network, the deterrence system can even be activated by remote operators, allowing them to monitor the protected area in real-time. The result is a complete system capable of providing an autonomous, non-lethal, and versatile solution for the protection of livestock in alpine areas.

To validate the system's performance and evaluate the effectiveness of the selected technologies, a prototype was developed and currently deployed in a semi-captivity facility for preliminary field testing to assess the deterrence's reliability. Following this validation phase, the prototype will be further refined and scaled to a network of multiple units deployed across grazing areas, establishing a virtual fence. This distributed approach aims to provide cost-effective livestock protection while avoiding the need for lethal control measures.

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A Dual Triggered Smart Detection System based on RADAR and PIR for Wolf-Livestock Conflict Mitigation

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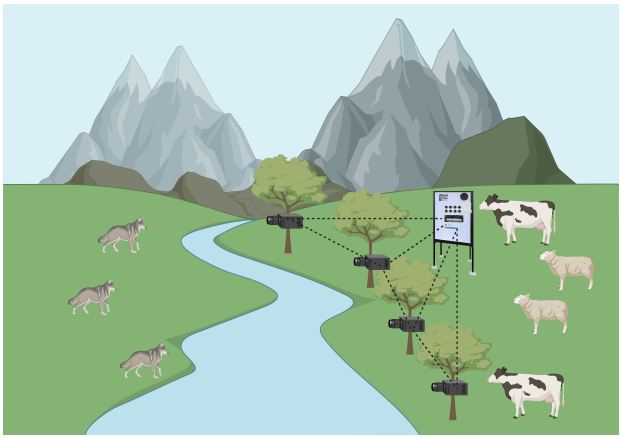


Figure 1: Schematic of the proposed dual-triggered smart detection system deployed in a representative pastoral scenario. Multiple sensing nodes are distributed around a livestock grazing area to detect the presence of wolves and activate the deterrence mechanism.

In northern Italy, several large mammalian predators, including wolves [1], bears [2] and golden jackals [3], have been progressively recolonizing, with populations increasing steadily in recent years [4]. This expansion has intensified human-wildlife conflict, particularly in rural and mountainous areas, where extensive livestock farming remains a key economic activity. Predation events occur throughout the year but are concentrated during spring and summer, resulting in significant economic losses for breeders and farmers [4].

Over the last decade, various deterrence systems have been deployed to mitigate livestock predation, yielding partial but insufficient results, as attacks continue to occur despite these measures. There is therefore a growing need for reliable, accurate, and energy-efficient detection systems capable of identifying the presence of large carnivores in proximity to grazing areas, enabling timely alerts and early intervention to prevent attacks. Such systems should be suitable for deployment in remote and off-grid environments [5], where traditional monitoring approaches are often impractical.

In this work, we propose a smart detection system capable of detecting and identifying large predators, particularly wolves, at long distances. The system integrates a dual-trigger mechanism based on radar and Passive Infrared (PIR) sensors, designed to minimize false positives by activating only upon the detection of heat variations. Once the dual-trigger system is activated, a camera is exploited to take images and videos of the detected target, also during night thanks to the infrared features of the camera. The acquired data is then processed by a machine learning classification model, which provides a binary output indicating the presence or absence of wolves. The classification model is based on a lightweight convolutional neural network optimized for embedded deployment, enabling real-time classification with low power consumption. Fig. 1 reports, on the left, the prototype deployed at the semi-captivity test site and, on the right, a night-time and daytime pictures taken by the system's cameras.

The proposed system represents a promising tool for enhancing human-wildlife coexistence by combining efficient detection with targeted deterrence, while minimizing environmental impact, operational costs, and potential livestock losses.

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Field Testing of an Acoustic Anti-Wolf Collar for Livestock Protection in Southern Italy

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The recolonization of the wolf in Italy has intensified conflicts with extensive livestock farming and increased pressure on traditional pastoral systems [1], [2]. This contribution presents field results from a pilot study conducted in Bova Marina (Reggio Calabria, Southern Italy) to evaluate an acoustic anti-wolf collar as a complementary non-lethal deterrent. From June to August 2024, 150 Aspromonte goats were divided into three groups of 50 animals managed under different protection regimes: shepherd only (SO), shepherd with livestock-guarding dogs (SGD), and shepherd with livestock-guarding dogs plus one anti-wolf collar mounted on a sentinel goat (SGDC).

The device emits modulated acoustic phrases built from natural harmonic intervals, using discrete fundamentals between 105 Hz and 1200 Hz. The prototype was designed for continuous field operation, with an effective range of approximately 50–100 m depending on topography and climatic conditions [3]. During the trial, camera traps confirmed persistent wolf activity in the study area (54 images at CT1 and 42 at CT2), enabling comparison between predator presence and predation outcomes across the three treatments.

Table 1 - Predation outcomes recorded during the June-August 2024 field trial.

Group	Protection strategy	Attacks	Kills
SO	Experienced shepherd only	72	5
SGD	Shepherd + livestock-guarding dogs	53	1
SGDC	Shepherd + guarding dogs + 1 acoustic collar	0	0

Marked differences emerged between the three management systems. The SO group recorded 72 attacks and 5 confirmed kills; the SGD group reduced attacks to 53 and limited mortality to 1 goat; the SGDC group recorded no predation events despite continued wolf presence near the herd. Average daily milk yield during late lactation was also higher in SGDC (30 L/day) than in SO and SGD (26 L/day), suggesting an indirect welfare benefit associated with reduced predation risk. These preliminary findings support the integration of acoustic deterrent technology with shepherding and guarding dogs as a promising strategy for reducing wolf-livestock conflict and improving coexistence in Mediterranean pastoral systems [2]-[4].

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Molecular Measurement Workflows for Tick-Borne Pathogen Detection in Sub-Saharan Livestock

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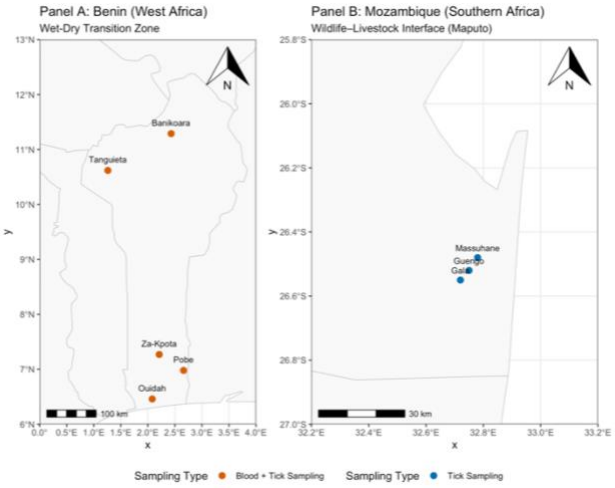


Figure 1 - Geographic distribution of sampling sites in Benin and Mozambique used to evaluate the standardized molecular measurement workflow

The reliability of veterinary diagnostics is fundamental to animal health monitoring and food security. Tick-borne piroplasms (*Theileria* and *Babesia* spp.) pose significant threats to livestock in Sub-Saharan Africa, yet diagnostic accuracy is often challenged by PCR inhibitory substances existent in blood (e.g. heme) or tick tissues (e.g. chitin), low parasite loads, and the lack of standardized analytical thresholds [1]. This study evaluates a standardized molecular measurement workflow for piroplasm detection in cattle blood and tick vectors across Benin and Mozambique (study areas shown in Figure 1), integrating real-time PCR screening with predefined decision thresholds and confirmatory conventional PCR/sequencing.

Between 2021 and 2024, piroplasms were investigated in 69 bovine blood samples and 245 ticks from Benin, and 326 ticks from Mozambique. To reduce PCR inhibition, an optimized extraction protocol featuring extended proteinase K digestion and modified binding conditions was utilized for tick samples. All samples were screened by real-time PCR targeting the LSU rRNA gene. Samples with Ct ≤ 34 and a melting temperature (Tm) between 74 to 76°C were subjected to end-point PCR targeting the 18S rRNA gene and Sanger sequencing for species-level identification. In Mozambique, a specific nested PCR targeting the p104 gene was applied to detect *Theileria parva*, given its known circulation in southern Africa and absence in Benin [2], [3].

In Benin blood, piroplasm positivity at real-time PCR was very high (60/69), and the ones reaching the analytical threshold were identified as *T. velifera* 58.0% (40/69) and *T. mutans* 8.7% (6/69) at the end-point PCR. In Benin ticks, real-time PCR screening yielded 52 positive specimens among 245 analyzed ticks. However, only a small proportion of these reached the analytical threshold, resulting in single confirmatory identifications of one *T. velifera* and two *Babesia bigemina* infected ticks. This marked discrepancy between screening positivity and confirmatory yield indicates reduced analytical efficiency in tick-derived samples. In Mozambique, real-time PCR indicated a high piroplasm signal burden (62.6%, 204/326 overall; up to 84.4% in *R. appendiculatus*), yet confirmatory 18S nested PCR yielded only 15 positives (4.6%; 4/15 successfully sequenced), evidencing againvery limited efficiency. In contrast, the species-specific p104 assay proved more resilient and highly effective, detecting *T. parva* in 12 ticks (11 sequenced).

These findings confirmed that Ct/Tm-based screening is a robust primary measurement tool, though the higher confirmatory rate achieved with the p104-specific PCR highlights the advantages of species-focused molecular detection over broad-range amplification in complex samples, such as the tick specimens. This standardized measurement framework can support reliable surveillance of tick-borne pathogens across different ecological settings.

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A Wearable Vibration System for Communicating with Dairy Cow

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Understanding and quantifying affective states in livestock is essential for welfare assessment and precision management, yet cannot rely on self-report and thus requires validated perceptual proxies[1]. Wearable technologies provide new opportunities for delivering controlled stimuli[2], but the feasibility of using vibration-based tactile signaling in dairy cows remains largely unexplored.

Two experiments were conducted at the Volcani Center experimental dairy farm to evaluate cows' perceptual and behavioral responses to vibration stimuli delivered via wearable devices. In Experiment 1, cows received standardized vibration pulses applied either to the neck or the leg, reflecting common locations of commercial monitoring systems, and behavioral responses (ear, neck, and eye movements) were quantified using predefined ethograms. In Experiment 2, cows were exposed to three temporally distinct vibration patterns ("words") of equal duration via neck-mounted devices, and behavioral activity during stimulation and control periods was compared to assess discrimination of signal structure.

Across both experiments, cows showed clear and repeatable increases in ethogram-defined behavioral reactions during vibration periods relative to non-stimulation controls, indicating reliable perception of the tactile cues. No significant differences were detected between neck- and leg-mounted devices, nor among the three vibration patterns, suggesting comparable detectability across body locations and signal structures under the current conditions. The absence of differential responses between vibration "words" implies that either the patterns were not perceived as functionally distinct or that the behavioral metrics lacked sensitivity to subtle discrimination.

The study demonstrates that dairy cows can robustly detect vibration-based tactile stimuli delivered via wearable devices at both neck and leg positions, establishing a critical perceptual prerequisite for tactile communication systems in precision livestock farming. While discrimination among unreinforced vibration patterns was not evident, pairing specific patterns with functional outcomes (e.g., rewards or management events) is expected to reveal associative learning and signal-specific responses. These findings provide a methodological foundation for future work on tactilely mediated communication, cognition, and welfare monitoring, including larger cohorts, reinforcement-based paradigms, and longitudinal assessments of habituation and preference formation..

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Sensor-based detection of drinking behaviour and water intake variability in dairy cattle for precision farming

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Water constitutes 60-70 % of the body of dairy cows and is a fundamental nutrient with multiple key roles like thermoregulation, milk synthesis, and metabolism support. Cows integrate water mainly through drinking, while losses occur via milk secretion, feces, urines, and evaporations. Various factors can influence water dissipation, including heat stress, with losses equal or greater than 20% being considered as fatal for this specie. For this, supplying water to dairy cows on a regular basis is needed to prevent any health and performance losses, especially during lactation.

In our study, we focused on the time window from 11:00 a.m. to 5:00 p.m. (warmest hours) and we evaluated the effect of the temperature-humidity index (THI) on variables like water intake, body temperature, number of bouts and time spent drinking in the monitored time window.

Six tie-stall Holstein-Friesian cows were monitored from 28 April 2025 to 2 July 2025 at the University of Bologna's experimental farm. Body temperature was recorded every 10 minutes via reticular bolus, and *ad libitum* water intake was measured every minute through individual drinkers.

The model adopted for the analysis of variance included cow ID and THI class as fixed effect, having considered three THI classes: THI value $\leq 10^{th}$ quantile (LOW), THI value between 10^{th} and 90^{th} quantile (MEDIUM), and THI value $\geq 90^{th}$ quantile (HIGH). The influence of THI was significant on all variables analyzed i.e., water intake, number of bouts, time spent drinking and body temperature.

In particular, water intake was highest in the HIGH class (54.6 ± 2.13 l) and lowest in the LOW class (50.1 ± 2.13 l), with a significant increase from LOW to MEDIUM ($p < 0.05$; 55.3 ± 0.76 l). Similarly, the least square means of body temperature, time spent drinking, and number of bouts showed significant ($p \leq 0.001$) increase with THI value. The results suggest that THI during the warmest hours of the day has a significant impact on the cow's drinking behavior and obviously in thermoregulation processes. Finally, Figure 1 shows that heat tolerance differed across cows and in some occasions the temperature was far above the cattle physiological threshold (39.2°C), indicating that individual variability in heat tolerance exists.

The work shows that heat stress can be effectively monitored through sensors designed on the individual physiology, enabling farmers to intervein in due time whenever early signs of heat stress or behavioral alterations occur.

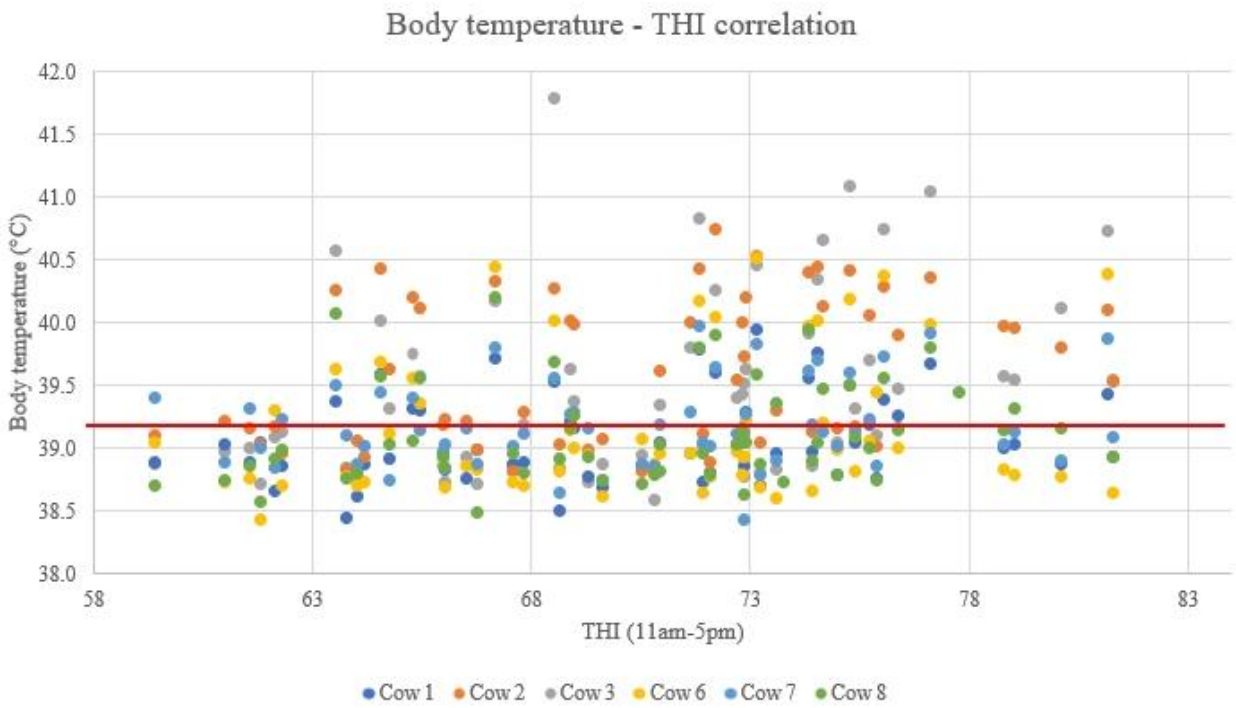


Figure 1. Scatter plot of THI vs. body temperature (11:00 a.m.- 5:00 p.m.) for each cow. The red line represents the upper physiological body-temperature threshold, beyond which the cow enters a state of hyperthermia.

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Does feed anticipation modulate vocal acoustic parameters in young water-buffaloes?

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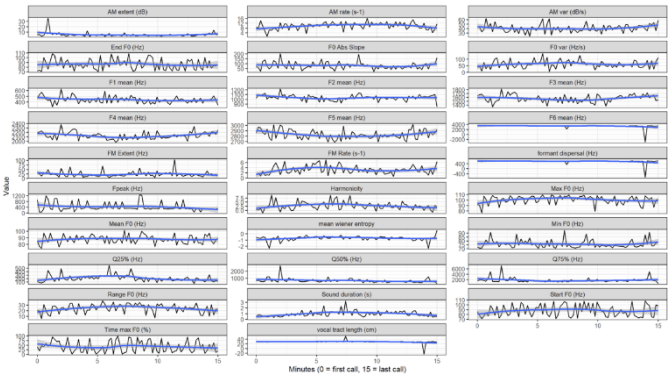


Figure 1 - Temporal dynamics of 29 acoustic parameters during the 15-min pre-feeding period in water buffalo vocalizations.

In large ruminants, predictable anticipatory states perceived by the animals as positive, such as feeding, induce effects in vocal production, especially to an increase in vocalization frequency. This study investigated whether vocal acoustic parameters are temporally modulated during the 15-minute period preceding concentrate feeding in young water-buffaloes. Our hypothesis was that vocal acoustic parameters are modulated as feeding time approaches. A total of 26 buffaloes (6–7 months old; both males and females) were mob recorded under routine management conditions. Vocalisations emitted within the 15-min pre-feeding window were collected (n = 84 calls). Recordings were obtained using a directional microphone (Sennheiser MKH416-P48U3; 40–20,000 Hz) connected to a digital solid-state recorder (Marantz PMD661). Acoustic analyses were conducted in Praat DSP v6.0.31 using an automated extraction script based on established source–filter methodologies [1–3], yielding 29 acoustic parameters per vocalization. Extracted variables included fundamental frequency (F0; perceived pitch) metrics, amplitude modulation (AM) and frequency modulation (FM) indices, spectral quartiles (Q25, Q50, Q75; frequencies below which 25%, 50%, and 75% of the spectral energy are distributed), formant frequencies (F1–F6; resonant frequencies of the vocal tract), harmonicity (harmonic-to-noise ratio), Wiener entropy (spectral flatness), and call duration. Data analysis was performed using R statistical software (v4.2.2). Temporal trends across the 15-minute anticipation period were assessed using Spearman rank correlations between call order (1–84) and acoustic parameters, complemented by linear slope estimation (change per minute). As individual identity was not tracked, repeated vocalisations from the same animals could not be accounted for, and observations may not be fully independent. However, given the focus on group-level temporal dynamics during anticipatory feeding, analyses were conducted at the call level. Results revealed a significant negative temporal trend for median spectral frequency (Q50) ($\rho = -0.377$, $p = 0.0004$; slope = −22.22 Hz/min), corresponding to an approximate 330 Hz downward shift in spectral energy distribution across the anticipation interval. This indicates a progressive redistribution of acoustic energy toward lower frequencies as feeding time approached. Additionally, call duration showed a positive association with time ($\rho = 0.238$, $p = 0.029$), indicating slightly longer calls closer to feeding. Nominal trends were observed for the sixth formant frequency (F6 mean) and lower spectral quartile (Q25), though these effects were non-significant. In contrast, most source-related parameters, including mean F0, maximum F0, F0 range, and FM measures, did not show consistent temporal modulation. These findings suggest that feed anticipation in young water buffaloes is associated with selective spectral redistribution rather than broad modulation of pitch or temporal structure. The progressive decrease in Q50 indicates that vocalisations become spectrally lower-weighted as feeding time approaches. While vocal signals show limited global restructuring, specific filter-related features may provide novel insights into the acoustic expression of anticipatory behaviour. Further studies integrating behavioural and physiological measures are needed to determine whether this spectral shift reflects arousal, motivational change, or expectancy-related modulation.

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Water-to-gain balance identifies distinct adaptation trajectories in young beef cattle imported from France

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The early post-arrival adaptation period represents a critical period for health and welfare of young beef cattle. This study monitored 106 young beef cattle during the first 30 days after arrival at an Italian commercial farm, across four batches received at different times of the year, to evaluate whether the water-to-growth balance could identify distinct physiological adaptation trajectories and provide early warning signals of compromised adaptation. Individual daily water intake was automatically recorded using a high-precision flowmeter integrated into the drinker, while individual body weight (CIMA srl, Correggio, Italy) was measured at each drinking visit using a livestock scale installed beneath the drinker. The Cumulative Water-to-Gain ratio (CW:G) was calculated as litres of water consumed per kilogram of body weight gained during the adaptation. Using the Youden J index, a threshold of 74 L/kg was identified: animals below this value showed a higher probability of receiving antimicrobial treatments. Combined with an average daily gain (ADG) threshold of 0.5 kg/day, five adaptation categories were defined: Failed adaptation (ADG and CW:G ≤ 0), Well-adapted (ADG $\geq$ 0.5; CW:G > 74), At-risk (ADG ≥ 0.5; CW:G ≤ 74), Delayed adaptation (ADG < 0.5; CW:G > 74), and Compromised adaptation (ADG < 0.5; CW:G ≤ 74). Factorial discriminant analysis integrating behavioural and physiological variables further characterised these groups. The Delayed adaptation group exhibited adequate hydration and low critical rectal temperature (RT) alarm frequency (>39.8°C) but slower growth, consistent with a progressive acclimation. The Compromised adaptation group combined reduced growth with inefficient water intake (WI) and frequent RT alarms, indicating limited coping capacity. The At-risk group maintained adequate growth despite lower WI and more frequent CT alarms: this performance was likely achieved under increased physiological strain, characterized by insufficient hydration and suboptimal compensation for thermal load. The Failed adaptation group displayed weight loss and marked physiological disturbance, with the highest RT values and alarm frequency. Animals of these previous categories were present across all seasonal batches and were not always associated with antimicrobial treatment. In contrast, Well-adapted animals exhibited high WI relative to growth and stable RT values, indicating effective thermoregulation and successful acclimation, mainly concentrated in the batch arriving in May and almost never treated. The water-to-growth framework identified dynamic adaptation trajectories rather than a simple healthy/diseased dichotomy, suggesting that clinical disease represents the endpoint of progressive maladaptation. Monitoring water-to-gain balance may therefore offer an early physiological indicator of compromised adaptation in newly received beef cattle.

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High-resolution monitoring of social interactions in dairy cows using a prototype Ultra-Wideband system

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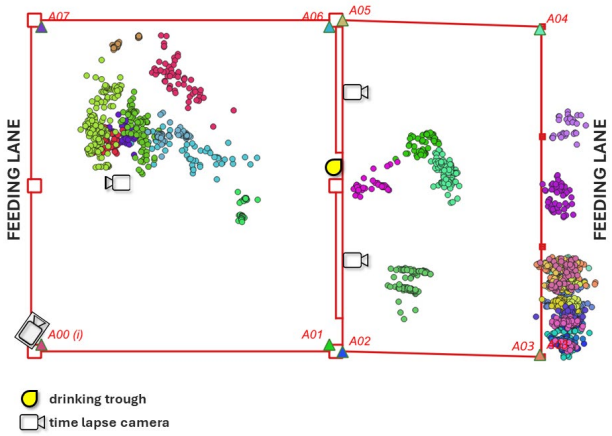


Figure 1 – Example for the analysis of UWB locations on a subset of data collected on 2 cows. Red lines: floor plan of the pen where the experiment was conducted. A00-A07: location of the UWB anchors. Clusters with dots of the same colour represent the location of a single dyadic interaction.

Social interactions significantly influence the welfare, health, and productivity of dairy cows. In intensive housing, sociality encompasses a spectrum of behaviours, from close proximity and physical contact to non-physical cues such as avoidance. The ability to detect these types of behavior is crucial for enabling automated systems for welfare evaluation, productivity optimisation, and early-stage disease detection [1]. Indoor tracking of individual animals poses unique challenges due to line-of-sight obstructions for sensor-based systems and visual constraints in camera monitoring. After a preliminary evaluation under controlled conditions [2], a prototype Ultra-Wideband (UWB) system was selected for a field application on a commercial farm.

This study aimed to validate the prototype system for indoor positioning of animals, to study inter-individual proximity and spatial utilisation patterns in permanently housed livestock. The system was composed of 12 prototype sensing units fitted on collars, which included a UWB module operating as a mobile node, a microcontroller, a real-time clock and an SD card, plus 8 fixed nodes (anchors). Absolute position data were collected for 6 consecutive days, at a sampling rate of 1Hz. Simultaneous video recordings were obtained using 4 time-lapse cameras covering the entire experimental area, recording 1 frame every 3 seconds. The twelve Holstein cows were housed in a pen, which included permanent straw bedding on the entire area, a drinking trough and an external covered zone (total surface 153 m^2). Data were processed in a GIS environment. Video recordings were utilised to establish empirical inter-individual distance and temporal thresholds for defining contact events. These thresholds were used to filter UWB data, obtaining clusters of dyadic interactions (Fig. 1). Further, the frequency of positions in the feeding, resting and drinking area was obtained for each cow. Spatial distribution was quantified through Kernel Density Estimation and spatial autocorrelation analysis to evaluate the occupancy of feeding, resting, and drinking functional areas. The detection of proximity events using the UWB system showed strong alignment with camera-based monitoring. The integration of high-resolution positioning with spatial statistics allowed for a quantitative assessment of how cows distribute within the barn. The experiment demonstrated the potential for UWB technology to provide continuous, automated welfare evaluation by tracking social dynamics and resource utilisation in real time.

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Exploring Natural Antimicrobial Strategies against *Campylobacter* spp. with Diverse Antimicrobial Resistance Profiles along the Poultry Production Chain

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Campylobacteriosis is the most frequently reported foodborne gastrointestinal infection in the European Union, according to the European Food Safety Authority (EFSA), and thermotolerant *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, represent its leading cause in industrialised countries [1]. Poultry meat is widely recognized as the primary vehicle of transmission to humans, with consumer risk mainly associated with the high prevalence of the pathogen along the production chain and with cross-contamination events between raw poultry meat and ready-to-eat foods. Furthermore, there is a growing consumer demand for sustainable and environmentally friendly strategies throughout the food chain. In this context, the aim of the present study was to assess the anti-Campylobacter and antioxidant activities of four polyphenolic extracts derived from olive mill vegetation water, an abundant agro-industrial by-product. *Campylobacter* isolates obtained at the retail level in central Italy were identified at the species level and characterized for their antimicrobial susceptibility profiles, providing insight into the dissemination of antimicrobial-resistant strains along the production chain [1]. The extracts included two liquid formulations (AgriPhen and Phen 50L) and two encapsulated formulations (Phen 30+ and Phen 50W). Preliminary, chemical characterization was performed using liquid chromatography–quadrupole time-of-flight (LC-QTOF) mass spectrometry, confirming the presence of several bioactive phenolic compounds, including hydroxytyrosol and tyrosol, along with minor amounts of vanillic acid. The average concentrations across extracts were 24.01 (8.12–40.68), 6.13 (2.94–9.10), and 0.47 (0.25–0.80) mg/g, respectively. The antioxidant capacity was evaluated using DPPH, ABTS, and FRAP assays, revealing variability among the extracts [2]. Mean values were 214.3 (110.3–466.4) µM Trolox equivalents/g extract for ABTS, 182.5 (62.5–319.3) µM Trolox equivalents/g extract for DPPH, and 188.2 (87.4–322.1) µM Trolox equivalents/g extract for FRAP, indicating overall high antioxidant activity across all formulations. The antibacterial activity of the extracts against *C. jejuni* and *C. coli* strains with different antimicrobial resistance profiles was assessed using agar well diffusion, microdilution, and time–kill assays. The results demonstrated that the extracts inhibited *Campylobacter* growth to varying degrees, likely depending on their chemical composition; moreover, encapsulation appeared to slightly reduce antimicrobial efficacy. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values ranged from 15.6 to 31.3 mg/mL for powdered extracts, while for liquid extracts they corresponded to dilutions between 1:32 and 1:64. Overall, these findings highlight the potential of olive mill by-products as natural and sustainable antimicrobial agents with diverse applications along the food chain, including use as feed additives, cleaning agents, and components of innovative active packaging systems. However, further studies are warranted to evaluate their effectiveness in real food systems and to assess their potential as alternative strategies to reduce Campylobacter contamination in poultry products, thereby enhancing food safety.

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Development of a Spectral Library for the Detection of Nitrite Fraud in Tuna

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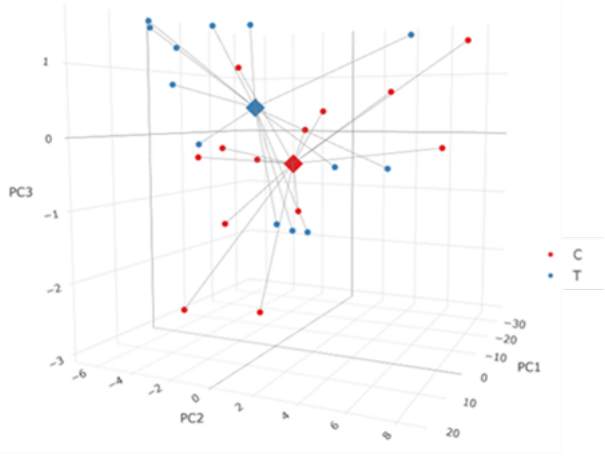


Figure 1. Principal component analysis of tuna samples: control (C) and treated (T).

Near-Infrared Spectroscopy (NIRS) is proposed as an untargeted analysis method combined with chemometrics analysis to evaluate different features related to food authentication. Robust libraries and classification models could be an important innovation for the fishery sector, providing a fast and easy approach for fraud detection. In this context, particular attention has been paid to practices aimed at altering product appearance and shelf life to deceive consumers. Nitrite salts, added to fish meat, alter the colour to mask the catch date or sell at higher prices, with microbial growth inhibition. The European Commission banned these treatments [1] to mitigate fraud and health risks due to the carcinogenic nitrogenated compounds formation [2]. Moreover, the addition of unapproved substances may alter the product matrix, leading to changes in spectral features that enable discrimination of adulterated products. This study aims to evaluate nitrite detection performances with a portable tool used in tuna (*Thunnus albacares*) fished in the FAO 71 area and artificially inoculated. From thirteen fresh tuna, one steak per fish was collected and divided to obtain paired control and treated samples. Each treated slice was supplemented with a 0.1% (w/v) $NaNO_2$ solution by syringe subcutaneous injection and stored at 4 °C for 48 hours to homogenise the distribution [2]. Each sample was analysed using both a NIRS portable instrument (900-1680nm range with 2nm resolution) and a colorimeter to capture colour and absorbance changes information [2,3]. The acquired spectra data were processed using supervised and unsupervised chemometric analysis. Classification and prediction analysis were performed using different Support Vector Machine methods applied to the raw spectral data. Moreover, the most significant wavelengths were identified using Boruta algorithms. Figure 1 represents the graphical distribution of the data visualised with principal components analysis, highlighting a good clustering of the data with 99% explained variance, despite a slight overlap. The most promising approach achieved an accuracy of 92%, which was statistically significant (P-value 5.245e-06). Moreover, sensitivity and specificity values were high, 100 and 85, respectively [4]. The features selected from the colorimetric analysis mainly refer to the redness (a*) and greenness (b*) coordinates under standard illumination, together with wavelength bands associated with browning phenomena and perceived lightness (e.g. 410, 450, 570 and 640 nm). These findings support the hypothesis that portable NIRS instruments are a rapid in situ tool for competent authorities with promising results for fraud detection involving low levels of adulterants and food preservatives.

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Inactivation of Opistorchis felineus metacercariae in tench by freezing and combined treatment with smoking and refrigeration

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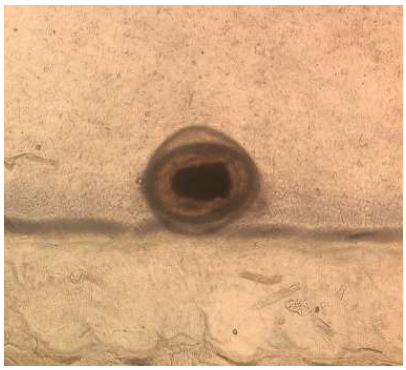


Figure 1 - Metacercaria of *Opisthorchis* spp. observed in tench (Tinca tinca) fillet following artificial digestion of pooled muscle samples. Observed under a stereomicroscope

Despite their recognized nutritional value, fishery products may act as vehicles for the transmission of zoonotic diseases, including those of parasitic origin [1]. Opisthorchiasis is a zoonosis caused by trematodes of the genus *Opisthorchis* and is transmitted through the consumption of raw or undercooked freshwater fish belonging to the family *Cyprinidae*. The principal definitive host is the cat; however, dogs, pigs, and rabbits may also serve as definitive hosts, whereas humans represent incidental definitive hosts. Within the muscle tissue of fish, the parasite develops into metacercariae, which constitute the infective stage for the definitive host. In Central Italy, particularly in volcanic lakes such as Bolsena, Bracciano, and Vico (Latium region), *Opisthorchis felineus* is endemic [2], and human outbreaks have been associated with the consumption of tench (*Tinca tinca*). A further outbreak of opisthorchiasis occurred in the Umbria region (Central Italy) in 2022 and was linked to the consumption of tench originating from Lake Bolsena. According to European food safety legislation, food business operators are required to ensure that raw materials and finished products undergo freezing treatments capable of inactivating viable parasites potentially hazardous to consumers. Specifically, *O. felineus* metacercariae present in the edible portions of fish can be inactivated either by cooking the product to a core temperature of at least 65°C for one minute or by freezing it at a core temperature of –20°C for a minimum of seven days [3, 4]. A local freshwater fish supply chain in Central Italy conducted an experimental study to mitigate this risk through freezing, hot smoking, and refrigerated storage treatments. Tench naturally infected with *O. felineus* metacercariae were selected and processed as follows: **Sample 0**: whole or filleted fish naturally infested with metacercariae (untreated control); **Sample 1**: fillets frozen at –26°C for 22 hours and subsequently stored at –20°C for 24 hours; **Sample 2**: Sample 1 fillets stored at –20°C for 3 days; **Sample 7**: Sample 1 fillets stored at –20°C for 6 days; **Sample S**: hot-smoked product (72°C for 1–3 minutes) subsequently stored at <4°C.Results, assessed on the basis of the presence or absence of viable metacercariae, demonstrated that untreated tench fillets (Sample 0) harbored viable parasites, whereas freezing under the tested conditions resulted in complete inactivation, even in heavily infested samples. The freezing protocol adopted in this study proved effective in eliminating the parasite, and the hot-smoking process applied was likewise sufficient to ensure metacercaria inactivation.

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Characterization of Goat Milk Cheeses from Native Breeds Produced Using Traditional and Innovative Ripening Methods

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Traditional cheesemaking practices, combined with local grazing practices that utilise the region's pasture resources, help establish a strong connection between dairy products and their region of origin [1]. However, to promote the diffusion of traditional products and support the conservation of native goat breeds, strategies aimed at improving process standardization and product valorization are required. This study aimed to evaluate the effects of breed and ripening method on the physicochemical, textural and sensory properties of cheeses produced from milk of Di Potenza (CP) and Derivata di Siria (DS) goats reared in Basilicata (Southern Italy). Two ripening methods were compared: a traditional system (TR; 26 °C, 50% RH) and an innovative controlled system (IN; 11 °C, 78% RH) using an industrial ripening device (Stagionello®; [2]). Raw milk was heated to approximately 65 °C and coagulated using vegetable rennet. Subsequently, the curd was manually collected and placed into perforated plastic moulds, where spontaneous whey drainage occurred. Surface dry salting was applied repeatedly in the morning and evening during the first day of maturation. Finally, cheeses were ripened for 30 days. For each cheese-making session, samples of raw milk, cheese on the first production day and ripened cheeses were collected. Significant differences were observed as a function of both breed and ripening method. During ripening, moisture and water activity decreased, with a corresponding increase in fat and protein content ($p < 0.01$) [2]. Colour parameters were influenced by the ripening methods, with TR cheeses showing greater yellowness (b*) at the end of ripening, while lightness (L*) decreased in all samples ($p < 0.01$). Texture profile analysis indicated an increase in hardness, gumminess, elasticity, and chewiness during ripening, with TR cheeses exhibiting higher hardness values than IN cheeses. Breed mainly affected lipid composition [3], whereas the ripening method influenced lipolysis and lipid oxidation, with DS cheeses showing the highest values after 30 days. Only minor changes were observed in the fatty acid profile. Analysis of volatile compounds showed distinct differences between ripening methods. IN cheeses exhibited higher sensor responses linked to sulfur compounds, nitrogen oxides, and methane compared to those made using TR methods. This indicates a significant impact of controlled environmental factors on the development of the aromatic profile. The results indicate that adopting measurable innovative ripening systems can improve quality consistency and standardization of cheeses from autochthonous goat breeds, while promoting livestock biodiversity and supporting the sustainability of local dairy production.

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Application of Electronic Nose technology to differentiate aroma profiles in bovine and buffalo cheeses subjected to various dietary treatments.

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Cheese aroma is a primary quality indicator influencing consumer perception, and it can be objectively evaluated using an analytical instrument (electronic nose) that combines artificial intelligence with ten sensors to discriminate samples and assess freshness and storage time [1;2]. In this study, an electronic nose was utilized to assess the aromatic profiles of mozzarella, scamorza, and caciocavallo made from bovine milk, along with the same three cheese varieties produced from buffalo milk.For each cheese type and animal species, control samples (CTR; cheeses obtained from milk of animals fed a conventional diet) were compared with treated samples (TRT; cheeses obtained from milk of animals fed a treated diet) to assess treatment-induced modifications in aroma. All samples were analyzed using the PEN3.5 electronic nose, and differences among samples were evaluated through pattern recognition analysis based on Principal Component Analysis (PCA), employing a score plot model. To better interpret the results, individual sensors associated with specific classes of volatile compounds were considered: S1 (W1C), sensitive to aromatic compounds; S3 (W3C), related to ammonia and early protein degradation; S7 (W1W), sensitive to sulfur-containing compounds associated with undesirable notes; and S8 (W2S), related to alcohols and lactic freshness. The PCA revealed a discrimination between CTR and TRT samples for all cheese typologies and for both animal species, indicating that the applied treatment significantly influenced the volatile compound profiles. Regarding the contribution of individual sensors to the PCA discrimination, in bovine cheeses, TRT samples exhibited slightly lower responses of S1 and S3 compared to CTR, suggesting a reduction in aromatic intensity and ammoniacal compounds. Conversely, sensors S7 and S8 exhibited slightly higher responses in TRT samples, indicating an enhanced lactic aroma profile, albeit accompanied by an increase in sulfur-related compounds. In buffalo cheeses, a different trend was observed for sensors S1, S3, and S7, as TRT samples showed higher responses of S1 and lower responses of S3 and S7 compared to CTR, suggesting a reduction in sulfur-containing and ammonia-related compounds. Sensor S8 exhibited the same trend observed in bovine cheeses, indicating an enhancement of lactic freshness and overall aromatic quality in TRT samples, in agreement with the increased response of S1. Overall, the results showed that the treatment's efficacy was species-dependent, with buffalo cheeses exhibiting a more pronounced treatment effect than bovine ones.
This study also demonstrated that measuring electronic nose enables the objective differentiation of aromatic variations among samples and provides guidance for future research.

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Repeatability of MALDI-TOF MS *Campylobacter jejuni* spectra in sub-species studies

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In literature most MALDI-TOF MS research focuses on bacterial species identification, while strain typing remains less explored and considerably more complex [1]. According to ISO 3534-2:2006, repeatability describes precision under repeatability conditions, namely the same measurement or test procedure, operator, measuring or test equipment used under the same conditions, location and repetition over a short period of time. Reproducibility refers to precision obtained when independent results are generated using the same method on identical samples but in different facilities and by different operators [2]. In this context, the aim of this work was to evaluate MALDI-TOF MS repeatability for subspecies identification.

For bacteriological analyses using MALDI-TOF MS, clear guidelines defining repeatability and reproducibility are lacking, and available literature provides limited indications. Some MALDI-TOF-MS guidelines refer to robustness, defined as a measure of the resilience of a method to changes in environmental or performance conditions. Sample preparation and extraction methods strongly influence mass spectral profiles [3]. In routine diagnostic direct colony transfers widely used because of its speed and simplicity [4]. Strain typing using MALDI-TOF MS requires significantly higher reproducibility than routine species identification, since differences between strains are often minimal and can be overlaid by technical noise [5].

A total of 90 *Campylobacter jejuni* strains previously collected from various sources were used to assess the repeatability of the direct colony transfer method by MALDI-TOF (Biotyper, Bruker). Each strain was analyzed on three different days using the same cultivation conditions and preparation protocols (direct transfer of a colony onto the target plate with the addition of HCCA matrix) and the same MALDI-TOF instrument by the same person. Spectra with identification scores with reliable species identification (more than 2.000) were imported into Clover BioSoft. Raw spectra and pre-processed spectra were tested using the reproducibility analysis module to evaluate the consistency and reliability of the spectral profiles. The average spectrum for each isolate was compared with the respective triplicate spectra, resulting in Pearson's coefficient and in the calculation of the differentiation index. Pearson's coefficient ranging from 1 to 0.90 was selected to indicate high within strain similarity since values close to 1 represents good concordance [6].

60% of the raw spectra and 93.3% of pre-processed spectra met this criterion. Findings highlight the need for expanded research on repeatability and reproducibility in MALDI-TOF subspecies studies. Further investigation is required to clarify how factors such as strain diversity, instrument characteristics, and laboratory organizational practices may influence strain typing performance beyond routine species identification.

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The longissimus dorsi ultrasound evaluations influenced by stocking density in beef heifers

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Greater stocking density may negatively affect beef cattle's growth. The longissimus dorsi ultrasound is a method for assessing their performances in vivo evaluating fat (intramuscular fat (IMF) and backfat thickness (BFT)) and muscle (muscle thickness (DEPTH) and ribeye area (RIBEYE)) components. European recommendations suggest a space from 2.4 to 5.5 m²/animal. However, a ≥13 m²/animal is suggested for heavy animals to reduce group stress. The aim of the study was to evaluate beef heifers housed at two stocking densities using longissimus dorsi ultrasound.

A total of 54 Charolais heifers were enrolled (protocol number of ethical approvals 17498/2024). Animals were randomly divided into indoor boxes at arrival composing: high-stocking density group (HIGH_D; 5.54 m2/animal, N=26) routinely adopted in the farm, and low-stocking density group (LOW_D; 10.3 m2/animal, N=28) as a compromise between animals' and farmer's needs. The longissimus dorsi ultrasound evaluations were performed at arrival (D0), and after 30, 60, 90 and 120 days (D30, D60, D90, and D120) until slaughtering. The animal body weight (BW) was measured at D0, D60, and D120 and average daily gain (ADG; Kg/day) was then calculated among time-points. Finally, the carcass yield was obtained by the slaughterhouse. Statistical analysis was conducted with linear mixed models, a $p \leq 0.05$ was used as significant, and $0.05 < p \leq 0.10$ as trend.

The LOW_D had greater final BWD120 (+15Kg; p=0.007). Additionally, the ADG differed only after mid-cycle (ADGD120_D60; p<0.001), with LOW_D exhibiting around 270gr/d greater ADG than those in HIGH_D. This divergence resulted in a greater overall ADG across the entire production cycle (ADGD120_D0; p=0.073). These findings suggested that the impact of stocking density on growth performance may become more evident as animal increase in size. Ultrasonography findings evidenced a greater DEPTH (+0.61-0.72 cm; p=0.021) and RIBEYE (+8-16 cm2; p<0.001) from D30 to D120, BFT (+0.19-0.33 cm; p<0.001) from D60 to D120, and IMF (+0.77-0.81%; p=0.015) from D90 to D120 in LOW_D. These results could be related to lower competitive feeding behavior, and greater animals' movement with an improvement in the musculoskeletal system development with the increase in space allowance. Consistent with this hypothesis, carcass yield was greater in the LOW_D group compared with HIGH_D (58.89% vs. 57.96%, respectively; p=0.023). In conclusion, this study confirmed that stocking density affects beef heifer performance and health with animals housed at 10.3 m²/animal had better growth outcomes, ultrasound fat deposition pattern and muscle growth.

UNet for automatic pneumothorax detection on canine and feline CTs

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Introduction
Pneumothorax is a clinical condition defined as the pathological presence of air within the pleural space. Multiple etiologies may account for its development, with traumatic causes being the most common in veterinary medicine, making pneumothorax a frequent finding in emergency settings [1]. Computed tomography (CT) is considered the gold standard for the detection of pneumothorax; however, prompt evaluation by a radiologist is not always feasible. Artificial intelligence (AI) in veterinary medicine has been successfully applied to lesion segmentation, and its implementation in human medicine for pneumothorax detection has already been explored [2–3].

Materials and methods
Data were collected from different facilities to develop an AI-based model for the automatic detection of pneumothorax in canine and feline CT images. Ground truth determination was obtained through manual segmentation of pneumothorax performed by a radiologist using the open-source software 3D Slicer. The nnU-Net v2 architecture was selected for model development. Model performance was evaluated using the Dice Similarity Coefficient (DSC), the Aggregated Dice Score Similarity Coefficient (DSCAgg), and the Average Symmetric Surface Distance (ASSD).

Results
One hundred CT scans of dogs and cats were retrospectively collected; 80 were used for training and 20 for testing. An additional 47 randomly selected negative cases from the same institutions were included as negative controls.
The model showed a DSC of 0.797, a DSCAgg of 0.9267, and an ASSD of 3.281 mm.

Discussion
A U-Net–based model for the automatic detection of pneumothorax in dogs and cats was developed. DSC values ranged from 0.43 to 0.95 and showed a positive correlation with lesion size. Despite this variability, the mean DSC indicates robust overall performance in detecting pneumothorax and delineating its extent. DSCAgg of 0.9267 demonstrates excellent overlap between predicted volumes and ground truth, mitigating the influence of smaller lesions on the overall evaluation. An ASSD value of 3.281 mm reflects good spatial accuracy, with minimal differences between predicted and actual lesion boundaries.

Conclusion
The present work represents the first report of fully automatic pneumothorax segmentation from CT scans in veterinary medicine. Accurate determination of the volume and distribution of free air within the thoracic cavity would be particularly valuable in emergency settings; automated CT image analysis could speed up radiological evaluation or offer a preliminary diagnosis when a radiologist is not immediately available, thereby representing a cost-effective and time-saving solution.

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Deep learning–based automated segmentation of canine adrenal glands nodules on CT-images

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Introduction

Canine adrenal gland disorders include hyperplasia and functional or non-functional neoplasms with important clinical implications [1]. Computed tomography (CT) plays a central role in adrenal evaluation; however, assessment of adrenal nodules remains challenging due to their small size, variable appearance, and proximity to other abdominal organs and major vessels [2]. In routine clinical practice, evaluation often relies on subjective assessment and linear measurements, while volumetric analysis is limited by the time required for manual, operator-dependent segmentation [2]. While deep learning approaches have enabled accurate automated detection and segmentation in human medical imaging [3,4], veterinary applications—particularly for adrenal imaging—remain limited [2]. The aims of this study were to develop and validate a deep learning–based pipeline for automated detection and segmentation of canine adrenal nodules on CT and to evaluate the feasibility and performance of an automated segmentation pipeline for quantitative adrenal assessment

Materials and Methods

This retrospective multicentre study included late-phase post-contrast abdominal CT examinations collected from Antech Imaging Services and the University Veterinary Teaching Hospital of Padua. A total of 125 dogs with mono- or bilateral adrenal nodules met the inclusion criteria. Two radiologists independently reviewed CT studies, generating reference standard annotations. Manual segmentation of bilateral adrenal glands, kidneys, abdominal aorta, and caudal vena cava was performed using 3D Slicer. An example of segmentation is reported in Figure 1. The dataset was divided into training (n=85), validation (n=15), and test (n=25) subsets. An additional cohort of 250 dogs without reported adrenal abnormalities was included to model normal adrenal anatomy. Model performance was assessed using Dice Similarity Coefficient (DSC) and surface-based metrics.

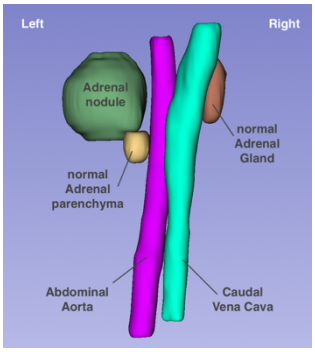


Fig. 1 Example of segmentation: adrenal nodule with normal adrenal parenchyma on the left; and normal adrenal gland on the right

Results

For left and right normal adrenal glands, Dice coefficients reached 0.82 and 0.72 respectively, with Average Symmetric Surface Distances (ASSD) of 0.52 mm and 0.72 mm respectively, indicating high contour accuracy. Across the combined dataset, the model achieved a Dice coefficient of 0.76 and an aggregated Dice (DSCAgg) of 0.84. The overall ASSD was 0.99 mm, reflecting submillimetric agreement with manual annotations. The 95th percentile Hausdorff Distance (HD95) was 5.18 mm, with higher values (HD95 of 10.10 mm) observed in nodular cases.

Conclusions

The proposed deep learning pipeline enabled accurate and reproducible automated segmentation of canine adrenal glands and adrenal nodules on CT. Automated volumetric assessment may support standardized and time-efficient quantitative evaluation of adrenal lesions, providing a foundation for future clinical and research applications in veterinary adrenal imaging.

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Development of an artificial intelligence-based method for the quantification of left atrial size in dogs from lateral thoracic radiographs

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Cardiac disease represents one of the most prevalent and clinically relevant conditions in dogs. Early detection of such conditions is crucial to guide therapeutic decisions and improve outcomes. Thoracic radiography should be performed as an early diagnostic tool in clinical practice if cardiac disease is suspected. Among radiographic indices, the Vertebral Left Atrial Size (VLAS) is considered an objective method to quantify left atrial size from lateral thoracic radiographs in dogs [1]. Artificial intelligence (AI) has emerged as a powerful tool to support clinicians in daily practice and automatic evaluation of the cardiac silhouette on thoracic radiographs can be useful in the early diagnosis of heart disease in dogs [2]. The aim of this study was to develop and validate a deep learning model for automated measurement of VLAS in canine lateral thoracic radiographs. A total of 427 canine latero-lateral thoracic radiographs were retrospectively collected. Ground truth annotations were manually performed by experienced observers. The following anatomical landmarks were identified: the cranial aspect of fourth thoracic vertebral body (T4), the caudal aspect of T4, the carina and the caudal margin of the left atrium. VLAS was calculated as the ratio between the distance from the caudal to the cranial aspect of T4 and the distance from the carina to the intersection between the caudal vena cava and the caudal left atrium. A deep learning-based neural network was trained to automatically detect these landmarks and compute VLAS. Agreement between automated and manual measurements was assessed using mean difference, Pearson's correlation coefficient (r), and Lin's concordance correlation coefficient (CCC). The automated system successfully identified the predefined anatomical landmarks across the dataset. The mean absolute difference between automated and manual VLAS measurements was 0.25 (minimum 0.0005, maximum 1.13). Correlation analysis demonstrated a Pearson's r of 0.7213, indicating good linear association. Agreement analysis yielded a Lin's CCC of 0.7038, reflecting substantial concordance between the automated model and expert annotations. This study demonstrates the feasibility of automated VLAS measurement using deep learning in canine thoracic radiographs. The proposed system shows good agreement with manual assessment and may serve as a decision-support tool for veterinary clinicians. Potential applications include large-scale screening for cardiac enlargement, standardization of radiographic reporting, integration into PACS systems, telemedicine workflows, and support in high-throughput clinical settings. Such AI-driven tools may enhance diagnostic consistency, reduce workload, and facilitate early detection of cardiac disease in dogs, fostering closer collaboration between veterinary medicine and biomedical engineering.

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Benchmarking Deep Architectures for Automated VLAS Measurement in Canine Lateral Thoracic Radiographs

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Introduction

Accurate and repeatable quantification of left atrial size on lateral thoracic radiographs is essential for objective monitoring of canine cardiac disease. The Vertebral Left Atrial Size (VLAS) index operationalizes this by combining vertebral scaling with airway-cardiac landmarks, yet manual measurement is time-consuming and observer-dependent. Therefore, it would be beneficial to automate the proposed by using artificial intelligence [1]. We present a technical benchmark of multiple deep learning families for automated VLAS computation from latero-lateral radiographs, comparing convolutional, compound-scaled transformer-based, and detector/pose-based pipelines.

Materials and Methods

We retrospectively analyzed 427 canine latero-lateral thoracic radiographs. Expert annotators labeled four landmarks: (1) cranial aspect of T4, (2) caudal aspect of T4, (3) carina, and (4) the caudal margin of the left atrium. VLAS was defined as the ratio of (A) the carina-to-left-atrium distance to (B) the T4 caudal-to-cranial vertebral body length measured in vertebral units. We evaluated five models: (i) ResNet18 and ResNeXt50 backbones with a heatmap regression head for multi-landmark localization, (ii) EfficientNetV2 backbone with a lightweight multi-resolution heatmap heads, (iii) Vision Transformer (ViT) using patch embeddings with a coordinate regression head (soft-argmax decoding), (iv) YOLO26 in keypoint/pose configuration to directly detect the four landmarks in a single shot, followed by geometric post-processing. All pipelines shared: intensity standardization, scale-preserving resizing, and targeted augmentations. Training used AdamW with cosine learning-rate decay and early stopping. The agreement endpoints were Pearson's r and Lin's concordance correlation coefficient (CCC).

Results

All architectures successfully localized the required anatomical landmarks and produced automated VLAS values within reasonable limits. Nevertheless, EfficientNetV2 achieved the highest overall accuracy, yielding the lowest mean absolute VLAS difference of 0.25 (range 0.0005–1.13) and demonstrating the strongest agreement with expert measurements, with Pearson's r = 0.721 and Lin's CCC = 0.704. ResNet18, ResNeXt50, ViT, and YOLO26 also produced stable measurements but showed larger variance, particularly in cases with low carinal contrast or ambiguous left atrial margins.

Table 1. Comparison of the deep architectures for the localization

Model	Pearson's r	Lin's CCC
EfficientNetV2	**0.721**	**0.704**
ResNet18	0.371	0.368
ResNeXt50	0.472	0.454
Vision Transformer (ViT)	0.452	0.429
YOLO26	0.349	0.326

Conclusion

This benchmark demonstrates that automated VLAS quantification is feasible across multiple deep learning paradigms. EfficientNetV2 emerged as the most accurate and robust architecture, balancing precision, stability across radiographic variability, and computational efficiency. YOLO26 remains advantageous for real-time applications, whereas transformer models offer scalability but at higher computational cost and higher data requirements. Future work will focus on dataset expansion, uncertainty modeling, and domain adaptation to enhance generalization across institutions and imaging systems.

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Region-Based Hierarchical Training for Nested Small-Structure Segmentation in Veterinary CT

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Introduction: Segmentation of small anatomical structures is challenging when lesions occupy only a minimal fraction of the image, causing extreme class imbalance and unstable optimization. Cascaded and coarse-to-fine strategies have been proposed to address this issue [1, 2]. In brain tumor segmentation, Fabian Isensee et al. showed that region-based training, i.e., optimizing hierarchical regions instead of independent labels, improves performance. This was later confirmed by Jun Ma and Jian Chen [3, 4]. Adrenal nodules in veterinary CT represent a nested small-structure problem: nodules are contained within already small glands, with high variability and limited soft-tissue contrast. Prior work suggested two-stage cascades to mitigate imbalance [2]. We therefore evaluate region-based hierarchical training within the nnU-Net framework [5] for adrenal nodule segmentation, testing whether constraining nodules to their parent glands improves stability and accuracy.

Materials and Methods: We analyzed 295 CT cases with annotations of left gland (1), left nodule (2), right gland (3), and right nodule (4); 20% (n=59) were held out for testing. Models were trained using nnU-Net (ResEncUNet-L, 32 GB GPU) in 2D, 3D low-resolution, and 3D full-resolution configurations. Two strategies were compared. The first pipeline used hierarchical region-based training: composite gland regions (1+2 left, 3+4 right) were optimized first, and nodules (2,4) were segmented only within their glands. The second pipeline used flat multiclass segmentation without hierarchy.

Results: Across all tested flat nnU-Net configurations, only the 2D model completed training. However, its segmentation performance was not statistically different from the hierarchical approach. The 3D flat configuration failed to converge completely and did not complete training, yielding a Dice score of 0.0 without any significant improvement in the loss value. These findings indicate substantial optimization instability in the non-hierarchical 3D setup. The hierarchical model, by comparison, trained successfully and achieved a mean Dice score of 0.75 with an ASSD of 1.37 for nodule segmentation with continued loss reduction during the training. The key distinction was training stability: the hierarchical formulation consistently converged, whereas the flat 3D configuration did not.

Conclusions: Region-based hierarchical training proved essential for stable optimization of nested small structures. Flat multiclass learning failed in 3D settings, highlighting instability when small labels directly compete with large background regions, even when employing additional sampling strategies. Constraining nodules to anatomically defined gland regions reduces effective class imbalance and provides spatial context. For CT pipelines targeting small intraparenchymal lesions, hierarchical co-segmentation should be considered a default strategy.

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Open-Set Recognition of Dairy Cows with Computer Vision and RFID

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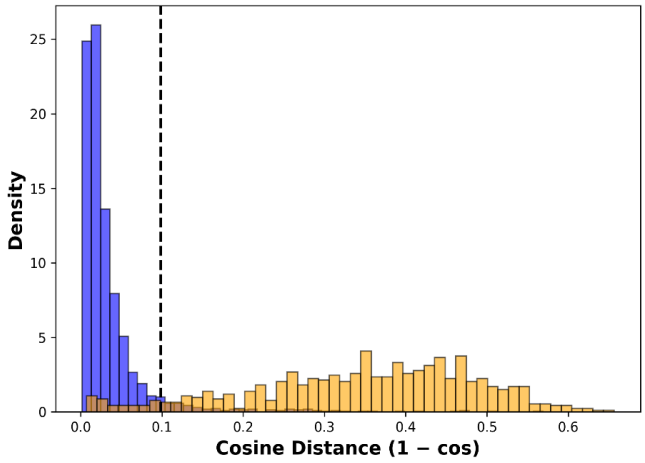


Figure 1 – Distribution of cosine distances from known class prototypes. Blue bars represent known individuals, while orange bars represent unknown individuals.

The aim of this work is to develop an automatic system for identifying dairy cows using computer vision, supported by RFID technology. Although RFID systems are widely adopted, they may suffer from missing data or incomplete coverage [1], [2]. In this context, computer vision can provide continuous identification [3], complementing RFID by reducing data gaps.

This paper addresses the problem of open-set visual identification of dairy cows using data acquired in a real farm environment with a single camera. Regions of interest corresponding to individual cows are extracted using an object detector. Moreover, a lightweight convolutional embedding network based on ResNet18 [4] is trained using metric learning with ArcFace loss.

The trial was conducted at the University of Bologna's experimental dairy barn on 41 Holstein cows equipped with RFID tag. After filtering, 29 identities were used for training and evaluation, while 12 were excluded and treated as unknown during testing. The dataset consisted of 9,374 training, 1,997 validation, and 2,036 test samples for known cows, plus 719 images of unknown cows for open-set evaluation. In the closed-set scenario, the methods achieved 97.89% accuracy and a macro F1-score of 0.98. In the open-set setting, the system effectively distinguishes known from unknown animals, obtaining a true acceptance rate of 0.95 (FRR = 0.05) and a true rejection rate of 0.96 (FAR=0.04).

The results demonstrate that metric learning allows reliable visual identification of dairy cows under realistic farm conditions. The integration of computer vision with RFID-based identification could improve the ability to track individual cows, contributing to improved precision livestock farming.

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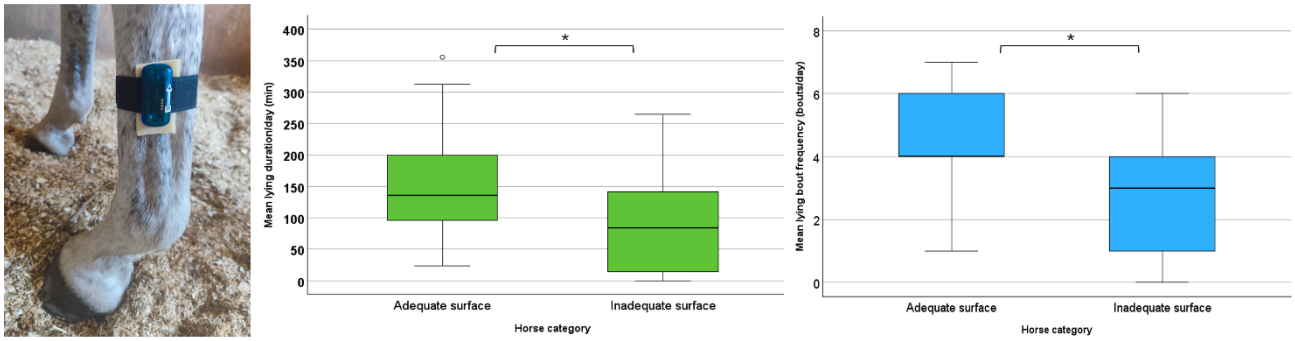
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The importance of having enough space: assessing lying behaviour in horses housed in single boxes

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Recumbency is fundamental for horses since it is the only way to achieve the necessary amount of REM sleep to avoid sleep deprivation [1]. Among the factors known to influence lying behaviour, the area of the shelter is particularly important, since horses kept for leisure and sport are often housed in single boxes, making it fundamental that the surface is enough to allow them to comfortably lie down and rest for a proper amount of time [2, 3].
The aim of the study was to monitor lying behaviour in horses kept in single boxes with adequate or inadequate dimensions related to the dimension of the horse.
The lying behaviour of 16 horses (10 mares and 6 geldings, 12.7±6.8 years old) of various heights at withers (mean±SD 158.5±13.5 cm) was continuously monitored for four days using triaxial accelerometers (MSR145 - MSR Electronics GmbH, Seuzach, Switzerland; Technosmart axy 5S - TechnoSmart Europe S.r.l., Rome, Italy) applied to the metacarpal bone of the left front leg [4]. Based on the ratio between the height at the withers and the box area [5], the subjects were divided into two groups: Adequate surface (A; N=8) and Inadequate surface (I; N=8). Generalized linear model was used to assess the influence of the suitability of the shelter area and the monitoring day on lying behaviour, specifically in terms of daily duration, frequency, and bout duration.
One horse of the Inadequate group never achieved decubitus during the monitoring period. The suitability of the area significantly influenced the mean lying duration ($p=0.001$) and the lying bout frequency ($p<0.001$), with a longer time and higher frequency for the Adequate group compared to the Inadequate one (A=154.4±13.9 min/day and I=90.9±13.7 min/day; A=4.5±0.3 bouts/day and I=2.5±0.3 bouts/day), while monitoring day did not seem to have an influence on lying behaviour ($p>0.05$).



a b c

Figure 1 *- a) Accelerometer placed on the left front leg (metacarpal region); b) Mean daily lying duration (min), across 4 days; c) Mean lying bout frequency (bouts/day), across 4 days*

Our results support the hypothesis that a larger available area promotes lying behaviour since the horse feels more comfortable in assuming a recumbent position [2, 3]. Furthermore, the triaxial accelerometer helps to non-invasively and accurately monitor lying behaviour in horses, allowing the identification of the absence of recumbency over a few days. Further research should aim to enlarge the sample size and consider different conditions where the use of a standard single box is required, such as in hospital settings or competitions.

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What we know about tools in horse sleep/resting behavior and why we should ask more

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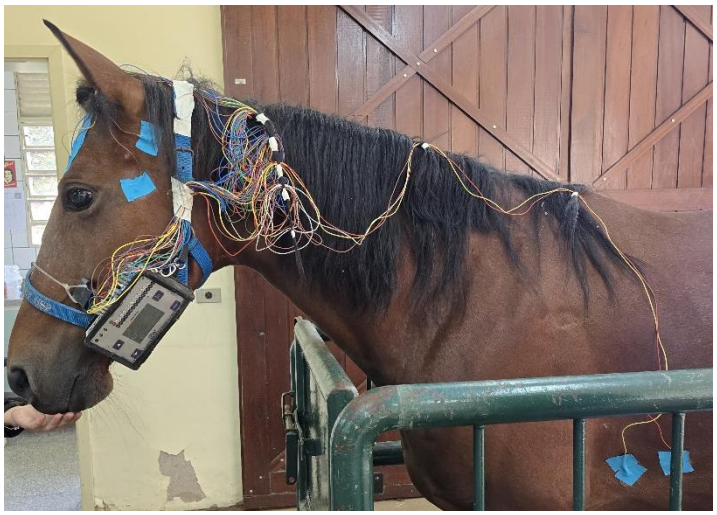


Figure 1 – Example of an equine using EEG.

Sleep is a reversible regenerative state of reduced responsiveness, and it is an essential part of welfare. Horses have polyphasic sleep pattern, having around three to four hours of sleep in a 24-hour period divided into two phases: rapid eye movement (REM) and Non-rapid Eyes Movement (NREM). There are some tools that can help sleep and rest behaviors identifications. The use of video cameras for monitoring stable is already established practice, using ethograms to analyze the equine resting behavior based on the positioning adopted by each animal within the stall [1]. The disadvantages are time-consuming analysis, space for media storage, and the uncertainty of the sleep quantity. In recent years, papers began to show applicability of deep learning-based modeling to make selected behavior detection almost instant [2]. This could also be applied to resting behaviors in the future as recumbency duration has been employed as an indicator of welfare, pain detection and environmental adaptation in horses [3,4, 5]. On the other hand, electroencephalography (EEG) (Figure 1) is an exam that assesses the electrical activity of the brain and has the potential to help identify in which sleep phase the horse is, just like a polysomnography exam in humans, being a gold standard to analyze sleep. It is a relatively innocuous exam, with the main disadvantages being the analysis time, stored data on long exams and the inconvenience for the patient. Hence, by discomfort, while it is possible to access the specific sleep phase, it could also be underrepresented [6]. Using frequency meter, it is possible to measure heart rate variability to monitor decrease or increase in heart rate during different sleep phases [7]. Additionally, there are accelerometers and gyroscopes. These devices can be used to detect recumbency, with the potential to differentiate between lateral and sternal recumbency, giving alternative of analysis resting behavior without cameras [8]. Following the measurements of accelerometers, actigraphy, used in sleep studies with humans, could be an alternative for equines as well to determine sleep patterns individually by monitoring for long periods. These devices can detect the presence of light, movement, and superficial body temperature [9]. The main disadvantage is not being yet validated for horses and not convey specific sleep stage. A wide range of technologies can be effectively used to advance the study of sleep and resting behaviors in horses, offering new insights into patterns and quality, aiming to improve the general welfare for these animals.

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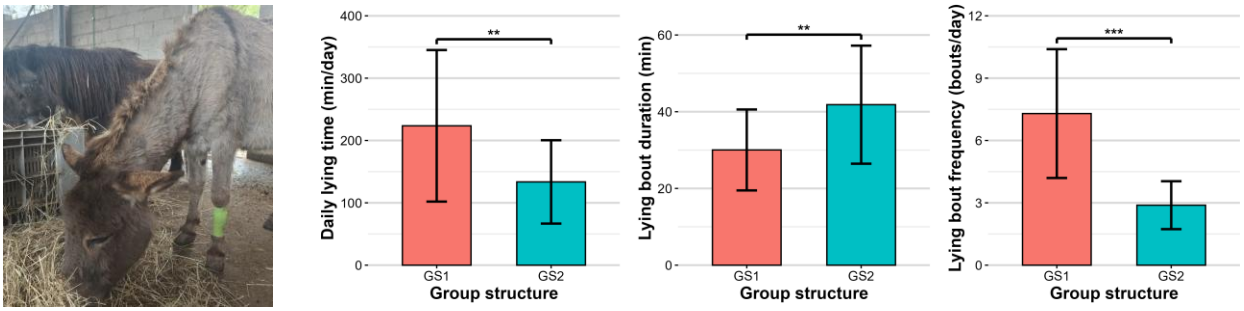
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How does social reshuffling influence lying behaviour in donkeys?

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Lorem Lying behaviour is a key component of animal welfare; however, quantitative information on its modulation by social organisation in donkeys remains scarce, despite the species' pronounced social nature [1]. This study investigated lying behaviour in domestic donkeys and assessed the influence of group structure, age, sex, and social affiliation across two observation periods characterised by regrouping (GS1 and GS2). Accelerometer data (Hobo Pendant) at 0.40 Hz were collected from 11 donkeys for two 4-day monitoring sessions, each providing continuous 24-hour recordings, from which daily lying time, lying bout duration, and lying bout frequency were extracted. At GS1, donkeys were housed in two stable social groups, whereas at GS2 all individuals were merged into a single group following social reshuffling. Effects of group structure, age, and sex were analysed using linear mixed models with donkey identity as a repeated factor. Daily lying time decreased from GS1 to GS2 following social reshuffling, from 223.6 ± 121.6 to 133.6 ± 66.9 min/day ($p = 0.003$), while lying bout duration increased from 30.0 ± 10.6 to 41.8 ± 15.4 min ($p = 0.007$) and lying bout frequency decreased from 7.3 ± 3.1 to 2.9 ± 1.2 bouts/day ($p < 0.001$). Age was significantly associated with daily lying time ($p = 0.023$) and lying bout frequency ($p = 0.006$), whereas sex had no detectable effect ($p > 0.05$). Lying synchrony was analysed at the dyadic level using linear mixed models with dyad as a random factor. Overall synchrony between all donkey pairs decreased from GS1 to GS2 (6.4% vs. 3.3%), in parallel with the reduction in mean lying time (15.97% vs. 9.60%). When considering socially affiliated dyads, synchrony was significantly higher under GS1 compared to GS2 (7.98% vs. 3.72%; $p = 0.005$). These findings indicate that lying behaviour in donkeys is sensitive to changes in social organisation and age, and that social reshuffling may disrupt coordinated lying among established affiliative partners, providing a foundation for future studies using larger populations and time-resolved behavioural measures.



a **b** **c** **d**

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Semi-Automated Assessment of the Ecotoxicological Effects of 3D Printing Materials on the Survival and Reproduction of *Folsomia candida*

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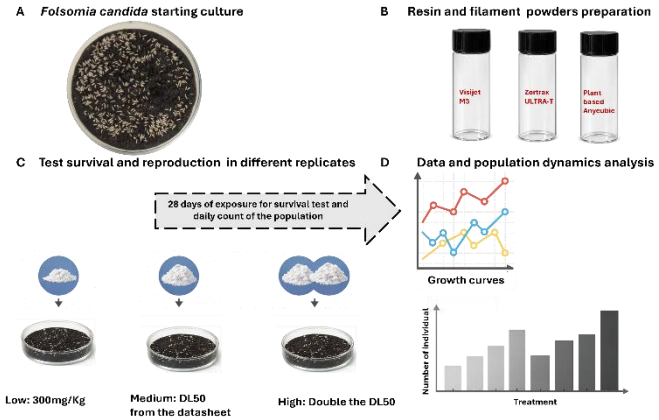


Figure 1 Workflow for the evaluation of the toxic effects on Folsomia candida after a chronic exposure. A starting culture in healthy condition (A), was used as source of adult individuals. The powder had been collected in separate vials (B) and tested in 3 different concentrations over 9-13 individual in replicates (C). After the 28 days of exposure numerosity were collected and analysed to describe the impact of 3D printing materials microparticles on springtails survival.

Additive manufacturing techniques have redefined engineering and medical industries, saving time and cost of production. However, their degradation may release microplastics (MPs) into the soil. Previous studies shown negative effects of MP on invertebrates' species, including springtails [1]. This study evaluates the effect of three materials tested as powders: Anycubic Plant-Based Resin (PB), VisiJet M3 Crystal (V3), and Zortrax Z-ULTRAT (ZU), on survival and reproduction of *Folsomia candida*. Standard ecotoxicological tests were conducted following OECD 232 protocol guidelines [2]. Tested concentrations were: PB: 300, 500 and 1000 mg/kg dry soil; V3: 300, 2000 and 4000 mg/kg dry soil; ZU: 300, 2000 and 4000 mg/kg dry soil. Concentrations were selected based on reported levels in low-contaminated industrial soils [3], the lowest LD_{50} from the resin datasheet, and twice the value. Preliminary results obtained using computer vision based semi-automated counting in ImageJ, revealed differences in reproduction and survival rates. The growth curves were not linear, showing an exponential growth after the eggs hatching. Relevant findings were described, the VisiJet resin showed a linear tendency regarding the averaged population abundance, with a relevant reduction of the survival rate when comparing the LD_{50} and 300 mg/Kg concentrations. While the most damaging compound resulted being the PB resin, which caused the highest mortality despite its natural origin. The highest tested concentration showed a reduction of about the two-thirds of living individuals with only one of three plates exceeded the 100 live individual thresholds set by the protocol. Also, the number of adults decrease for this material, meaning that the toxic impact affects both adults and newborns. For the ZU filament, results are not clear due to a non-linear trend in the response, with the highest concentration showing greater survival than lower concentrations. Further investigation would require potential agonistic or antagonistic effects of filament components, as well as possible hormesis and non-linear dose–response patterns in organisms exposed to MP. Given their industrial and economic relevance, further studies are needed to better understand the effects of chronic exposure [4]. Overall, further studies are needed to clarify possible long-term effects. This is particularly relevant for materials labelled as of natural origin, which may be perceived as sustainable due to their natural origin and employed without enough attention [5]. Our preliminary results, describe the effects of three 3D printing materials highlighting the necessity of further investigations, including ingestion assays and tests on non-extruded and non-polymerized material to assess environmental risks.

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Tech-Driven Evolution of Animal Housing: Exploring Technological Integration and Challenges in Mountain Livestock Farming

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The development and implementation of precision livestock farming (PLF) and smart technologies are increasingly critical to the profitability and sustainability of livestock farms [1, 2]. Within the dairy sector, these technologies are rapidly becoming sector standards [3, 4]. However, adoption patterns remain highly inconsistent, with significantly higher implementation rates in large-scale intensive systems compared to small-scale extensive farms, thereby creating a pronounced technological divide within the sector [5–7]. Alpine dairy farms are historically characterised by small-scale, family-operated farms strongly tied to niche PDO cheese production and the provision of environmental services, positioning them among operations with potentially lower PLF and smart technology adoption [10–12]. Despite this context, empirical knowledge regarding the actual implementation and potential of PLF in alpine dairy systems remains limited, particularly given the considerable variability across valleys and altitudinal gradients. This research aims to characterise the current state of dairy farming in the Alpine Province of Trento, with specific emphasis on housing infrastructure, PLF adoption rates, and smart technology implementation. This study synthesises data from public and private databases—including geospatial information and animal density metrics—with primary data collected through a comprehensive questionnaire administered to over 300 dairy farmers throughout the province, ensuring representative geographic and altitudinal coverage. Preliminary findings reveal substantial heterogeneity among alpine dairy farms and underscore critical context-specific factors that must be considered when evaluating future development pathways. Results will be presented through thematic spatial mapping to identify areas within the province where farms exhibit similar characteristics and requirements. Additionally, farmers' perceived priorities and investment intentions will be analyzed to conceptualize potential improvements and evaluate their feasibility relative to existing infrastructure constraints, ultimately providing evidence-based recommendations for the sustainable transformation of alpine dairy farming systems.

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Circadian collapse–recovery dynamics of physiological and behavioural signals improve estrous prediction in sheep using wearable sensors and AI-based modelling

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Accurate estrous detection remains a major challenge in sheep production systems, particularly when visual observation is impractical. This study integrates physiological and behavioral monitoring, circadian rhythm analysis, and artificial intelligence (AI)–based modeling to improve estrous (day 0) prediction in ewes using wearable sensors.

A total of 10 adult ewes were continuously monitored across the estrous cycle, yielding 15 complete estrous cycles for analysis. Estrus was objectively identified using vasectomized rams equipped with marking harnesses, providing a robust biological reference for defining day 0. Each ewe carried a subcutaneous temperature (T), heart rate (HR), and HR variability (SDNN and RMSSD) biologger (DST milli-HRT, Star Oddi, Iceland), surgically implanted, recording data at 30-min intervals. Locomotor activity (LA) was measured at 10-min intervals using a triaxial accelerometer (AX3, Axivity, UK) mounted on a collar.

First, the predictive value of raw absolute data was evaluated across days of the estrous cycle. All variables exhibited significant temporal modulation, with marked deviations around estrus. However, raw data were characterized by high inter-individual variability and limited robustness for precise estrous identification when used in isolation. To improve predictive performance, circadian rhythm analysis was applied using cosinor modeling on a day-by-day basis. For each variable, circadian parameters including mesor, amplitude, acrophase, and goodness-of-fit (R^2) were extracted. This approach revealed a consistent circadian collapse–recovery pattern centered on estrus. Specifically, the peri-estrus window (days −1, 0, and +1) was characterized by a transient disruption of circadian organization, reflected by reduced amplitudes, acrophase shifts, and diminished rhythm robustness. This collapse was followed by a progressive recovery of circadian structure during the subsequent luteal phase. The magnitude and timing of circadian disruption varied among variables. LA and T displayed clear attenuation of circadian rhythmicity around estrus, whereas autonomic-related metrics, particularly RMSSD, exhibited pronounced circadian instability, highlighting autonomic modulation as a sensitive indicator of reproductive status.

When cosinor-derived circadian parameters were integrated into AI-based predictive models, estrous prediction accuracy improved substantially compared with models based solely on raw data. Circadian features reduced noise, captured dynamic physiological reorganization across the follicular–luteal transition, and improved model generalizability across animals and cycles. Overall, LA and HRV-derived circadian metrics emerged as the most informative predictors. In conclusion, the integration of wearable sensor data with circadian rhythm analysis and AI-modeling provides a robust framework for estrous detection in sheep. The proposed circadian collapse–recovery model offers a novel physiological interpretation of estrous-related changes and supports the use of rhythm-based biomarkers for precision reproductive management in small ruminants.

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Autonomic Dynamics and Early Prediction of Parturition in Sheep Using Continuous Physiological Signals

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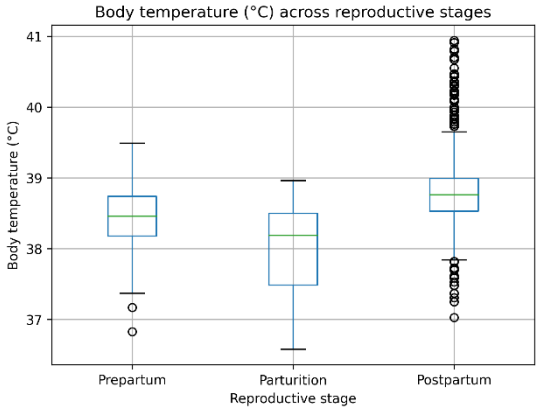


Figure 1: Distribution of body temperature, across reproductive stages (Prepartum, Parturition, and Postpartum). Boxplots represent the interquartile range with median values indicate.

Parturition in ruminants is a complex physiological process preceded by progressive endocrine, autonomic, and metabolic changes, regulated by the coordinated activation of the hypothalamic (pituitary) adrenal axis and the autonomic nervous system [1-2]. Early identification of this period is of major interest for reproductive management and animal welfare, as it enables optimized supervision of parturition and a reduction of risks for both dam and offspring [3]. The aim of this study was to characterize the physiological changes associated with parturition in sheep and to develop a predictive algorithm capable of anticipating the parturition period using continuous physiological signals.

Continuous physiological records were analyzed from **eight ewes** monitored for several days around the peripartum period. A total of **more than 2,700 valid observations** of body temperature (T), heart rate (HR), and heart rate variability (HRV) parameters (SDNN and RMSSD) were included and classified into **prepartum (n ≈ 1,200), parturition (n ≈ 40), and postpartum (n ≈ 1,500)** phases. Because the variables exhibited non-normal distributions and unequal sample sizes, differences among reproductive phases were evaluated using the **Kruskal–Wallis test**, followed by **post hoc pairwise comparisons with the Mann–Whitney U test**.

The parturition period was characterized by a decrease in body T (38.01 ± 0.62 °C) compared with the prepartum phase (38.46 ± 0.41 °C) (Figure 1), along with elevated HR values (135.8 ± 47.0 bpm) (Figure 2). Concurrently, a marked reduction in HRV was observed, with mean SDNN and RMSSD values of **29.5 ± 67.1 ms** and **38.7 ± 94.5 ms**, resp., during parturition, compared with higher values during the prepartum phase (SDNN: 35.2 ± 82.8 ms; RMSSD: 47.0 ± 121.4 ms). In the postpartum period, a significant autonomic recovery was evident, characterized by increased HRV (SDNN: 62.6 ± 115.7 ms; RMSSD: 87.5 ± 161.6 ms) and a reduction in HR (120.5 ± 56.1 bpm). The Kruskal–Wallis test revealed statistically significant differences among the three reproductive phases for all analyzed variables ($p < 0.001$).

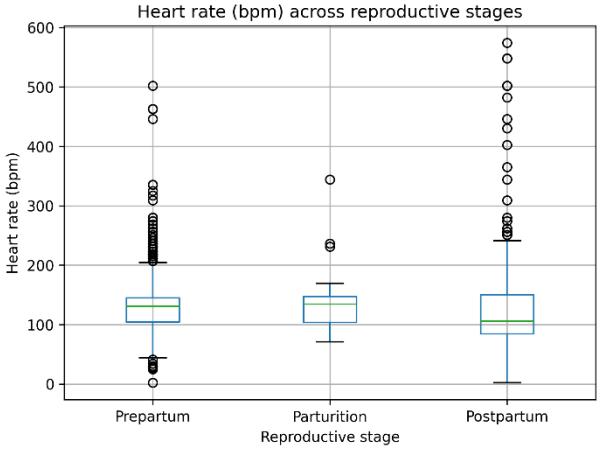


Figure 2: Distribution of heart rate across reproductive stages (Prepartum, Parturition, and Postpartum). Boxplots represent the interquartile range with median values indicate

Based on these findings, a predictive algorithm was developed incorporating intra-individual normalization of physiological variables, analysis of relative changes within temporal windows, and a probabilistic logistic regression model defined as

$$\boldsymbol{P(Parturition_t = 1) = 1/(1 + e^{-z_t})},$$

where z_t integrates relative changes in RMSSD, SDNN, HR, and body T.

This approach is supported by previous evidence identifying HRV as a sensitive biomarker of autonomic modulation and physiological stress in sheep [4]. Retrospective validation of the algorithm showed that, in all ewes analyzed, the parturition period was identified with **positive anticipation**, ranging from **11.8 to 126.3 hours** before the actual onset of parturition.

Overall, these results demonstrate the existence of an **autonomic signature of parturition in sheep**, characterized by progressive parasympathetic withdrawal and sympathetic activation prior to the clinical onset of labor. The findings support the use of statistical and algorithmic models based on HRV and continuous physiological variables as non-invasive tools for early parturition prediction and improved animal welfare.

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Use of bio-loggers to assess heat stress in *Piedmontese* cows under different stocking densities

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Heat stress is a major challenge for cattle welfare, particularly in housed systems, where management factors, such as stocking density, may influence animals' ability to cope with high environmental temperatures. Within the BESOS_RP research project, an integrated sensor-based approach was applied to assess environmental parameters, and behavioural and physiological responses of *Piedmontese* beef cattle related to naturally occurring heat stress conditions.
The study involved 20 adult suckler cows, housed at two different stocking densities: high density (HD: 3.5 m² of resting area per cow; n = 10) and low density (LD: 5.0 m² per cow; n = 10). Implantable subcutaneous bio-loggers were used for continuous individual monitoring of core body temperature and cardiac activity. At the same time, commercial collar-based sensors recorded behavioural and functional parameters, including rumination and an indirect index of respiratory response associated with heat stress. Monitoring was carried out from early April to early October. Barn environmental conditions (air temperature and relative humidity) were continuously recorded and used to calculate the temperature-humidity index (THI). In analogy, saliva samples were collected at standardized time points and analyzed for salivary cortisol quantification as an endocrine indicator of stress.
Focusing on the hottest period (summer period: June – September), increasing THI values were associated with clear behavioural changes. In particular, when THI exceeded the 75% alert threshold, rumination progressively decreased as heat load increased in both groups, confirming its sensitivity as an early indicator of thermal discomfort. HD cows showed higher respiratory response indices and lower rumination levels than LD cows. In contrast, although body temperature increased with THI, no marked differences in body temperature or heart rate were observed across stocking densities, suggesting a condition of compensated physiological adaptation. Salivary cortisol concentrations did not differ significantly between the experimental groups, with a similar trend throughout the trial.
Overall, these results indicate that integrating implantable bio-loggers and wearable sensors allows a detailed, continuous assessment of heat stress in *Piedmontese* cows. Behavioural indicators derived from sensors, particularly rumination and respiratory-related indices, appear to be more sensitive to heat load and housing conditions than baseline physiological or endocrine parameters. This approach represents a valuable tool for improving welfare assessment and supporting management strategies aimed at mitigating heat stress in beef cattle production systems.

The Connection Between Heart Rate, Accelerometry, and Metabolic Rate in Ungulates: Physiological Meaning and Applications for Biologging

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Quantifying energy expenditure and physiological stress in free-ranging animals is central to understanding animal health, welfare, and environmental adaptation. Heart rate (HR) has long been recognized as a reliable indicator of energy expenditure and autonomic nervous system activity, but continuous measurement over extended periods often requires invasive sensors, limiting its use in free-living animals. In contrast, accelerometer-derived metrics provide a non- or minimally invasive measure of activity and, in many cases, a proxy for energy expenditure. Acceleration collars are rapidly becoming a standard tool in biologging for large domestic mammals, including cattle and other ungulates. However, integrating these data to interpret how animals manage their energy budgets and particularly the relationships among resting metabolic rate (RMR), daily energy expenditure (DEE), and activity-related energy costs, remains challenging.

In mammals, minimum, mean, and activity-related heart rates have been shown to covary with proxies for RMR, DEE, and activity energy expenditure, although the strength and structure of these relationships vary within and among species [1]. These findings indicate that relationships between activity, heart rate, and metabolism are context dependent rather than governed by a single universal rule. Within this framework, accelerometry-derived metrics such as Overall Dynamic Body Acceleration (ODBA) and Vectorial Dynamic Body Acceleration (VeDBA) provide complementary measures of physical activity and mechanical workload. When interpreted alongside heart rate data, these metrics offer a mechanistic basis for linking externally measured movement to underlying physiological regulation of energy expenditure.

Recent work demonstrates consistent, though often non-linear, relationships between accelerometer-derived activity metrics and heart rate across behavioral states in large mammals [2-3]. Advances in modelling increasingly account for behavioral and environmental context and enable indirect estimation of energy expenditure using established cardiovascular–metabolic calibrations. In domestic species, where accelerometer collars are widely deployed, integrating activity and heart rate measurements provide a scalable pathway for remote assessment of energetic status and animal welfare. Continued refinement of calibration and modelling approaches, particularly those addressing behavior specific responses and non-metabolic influences on heart rate, will be critical for improving the interpretability and application of biologging data in veterinary and animal science contexts.

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Cardiac dynamics of mares during different breeding procedures: Mixed-effects models of HR and HRV using Polar sensors

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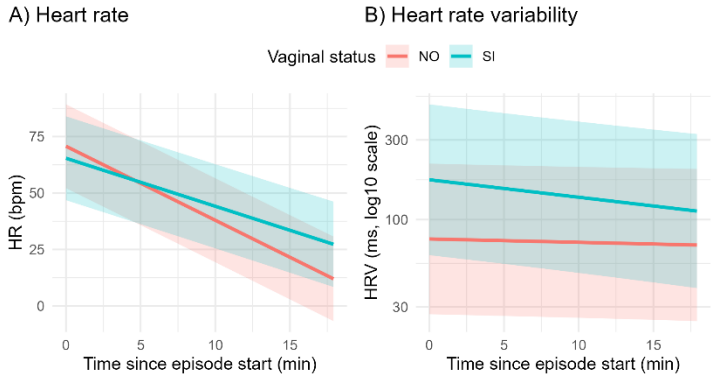


Figure 1 - Model-predicted (A) heart rate (HR, bpm) and (B) heart rate variability (HRV, ms; back-transformed from log scale) across time during breeding episodes, stratified by vaginal status (NO vs YES). Shaded bands represent 95% confidence intervals.

Breeding procedures can evoke acute autonomic responses in mares, yet objective, high-resolution evidence during routine reproductive management (natural cover and artificial insemination) remains limited. Heart rate variability (HRV) has been widely applied as a non-invasive indicator of autonomic regulation in animal welfare and stress research [1], and equine studies have shown that handling-related stimuli can shift HRV-derived autonomic balance [2]. In addition, Polar wearable systems have been validated against ECG for beat-to-beat interval recording in horses under ambulatory conditions [3].

Here, we quantified heart rate (HR) and Polar-derived HRV dynamics in mares during different breeding procedures and assessed the effects of vaginal manipulation and time within breeding episodes. Six mares were monitored at 1 Hz with Polar sensors during breeding events (three mares under natural cover and three under artificial insemination). For each second, we recorded HR (bpm), HRV (ms), time since episode start (s), and vaginal status (Vagina: YES/NO; presence of penis/insemination catheter). Episodes were defined by mare × estrus × repetition; timestamp discontinuities (>2 s) were used to split recordings into sub-episodes. Linear mixed-effects models included random intercepts for mare and sub-episode. In the models, β denotes the estimated fixed-effect coefficient, i.e., the expected change in the outcome per unit change in a predictor while holding the other predictors constant.

HR showed a clear time-dependent decline within episodes ($\beta = -0.0546$ bpm·s^{-1}), and this decline was attenuated when Vagina = YES (interaction $\beta = +0.0192$ bpm·s^{-1}), resulting in predicted HR values that were very similar between vaginal states around the mean episode time (Fig. 1A). HRV exhibited strong right-skew with extreme values; therefore, HRV was analysed on the log scale. At the mean episode time, Vagina = YES was associated with higher HRV ($\beta = 0.727$ on the log scale), corresponding to a little more than a twofold increase relative to Vagina = NO (Fig. 1B). A small negative Vagina×Time interaction ($\beta = -3.23\times10^{-4}$ log-units·s^{-1}) indicated a gradual decrease in HRV over time during Vagina = YES. Findings were robust in a sensitivity analysis after trimming the upper 1% of HRV values.

Wearable 1-Hz monitoring combined with mixed-effects modelling captured distinct, time-dependent cardiac patterns during breeding handling (Fig. 1), supporting the use of wearable biomarkers to objectively characterise acute physiological responses in equine reproductive management.

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Free-range hen activity with outdoor restrictions measured by tri-axial accelerometers

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Figure 1 – Hen with an accelerometer.

Free cage systems for egg production are increasing due to consumers' demands. Avian flu restrictions have been applied in Spain after the breakage that occurred in October 2025. These movement restrictions may have an effect on hen behaviour that have not been fully studied. The aim of this work was to assess circadian rythmn in free range hens that are forced to be indoor with a tri-axial accelerometer.

Twelve 70-week old hens, divided in two boxes (2x1m), with access to an exterior area (2.5x1m), carried an accelerometer (Axivity AX3; 23 x 32.5 x 7.6 (mm; 11 g) enclosed in a plastic box, and hold to the back by elastic bands (Figure 1) to record daily locomotor activity (LA) for 7 days. Initially, animals had free acces to the outdoor area. However, 48 h after the beginning of the trial they were forced to remain in the interior area without exterior access. Three periods have been assessed: Period 1 (48 h with free access to the outdoor park), Period 2 (24 h following enclosure, with no access to the park) and Period 3 (for 96 h after Period 2). Sensors were programmed to collect data at a rate of 30 Hz. The activity counts for the 3 axes were combined to create minute-by-minute activity Vector Magnitudes (VM). Circadian parameters of LA of each period, including MESOR, amplitude, and acrophase, were calculated. Statistical differences were assessed with an ANOVA with the period as the fix effect.

Data showed a clear effect of outdoor restriction on LA patterns in free range laying hens. Confinement induced an acute reduction in activity during the first 24 hours, reflected by lower MESOR (0.0197 vs 0.0218) and amplitude (0.0178 vs 0.0198) values. This initial drop indicates an immediate behavioral adjustment to the sudden loss of environmental complexity and space. However, after 24h, both MESOR and amplitude values were higher than those found in period 1. This probably reflected a nervious behaviour of animals that are accostumed to go outdoor and, due to sanitary restrictions, were not allowed to do it any longer. These findings support the use of wearable accelerometers as a reliable tool to monitor welfare-related behavioral changes in free range systems subjected to movement restrictions. Quantifying changes in MESOR and amplitude offers a practical framework to evaluate short-term adaptation and longer-term behavioral consequences of enforced indoor housing.

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Enumeration of Enterobacteriaceae in foodstuffs by BS EN ISO 21528-2:2017: is it time to change it?

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The family Enterobacteriaceae includes a heterogeneous group of Gram-negative proteobacteria which are ubiquitous with different ecological niches, colonizing humans, animals, insects, plants, water, and soil. Enterobacteriaceae is also one of the most taxonomically diverse bacterial families currently recognized, with recent taxonomic revisions for which several traditional genera moved into newly established families [1]. EC Regulation 2073/2005 on microbiological criteria for foodstuffs includes Enterobacteriaceae counts as process hygiene criterion [2]. They are used as indicator bacteria to provide evidence of poor hygiene, inadequate processing or post-process contamination of foods [3]. Relating to the slaughter of animals, they are also applied to evaluate enteric contamination of carcasses and as markers of pathogenic zoonotic pathogens coming from animals or processing environment [4].
BS EN ISO 21528-2:2017 specifies the reference method for the enumeration of Enterobacteriaceae in food products and environmental samples from primary production, food production and handling. At least five characteristic colonies (pink to red or purple, with or without precipitation haloes) must be subjected to biochemical confirmation tests [5]. Only oxidase-negative and glucose-positive colonies are confirmed and therefore count as Enterobacteriaceae.
The aim of this study was to evaluate the concordance between results obtained according to ISO 21528-2:2017 and the identification of the same isolates using MALDI-TOF.
A total of 52 samples, including food of animal origin (44.2%), plant origin (38.5%), environmental samples (5.8%) and unspecified sources from food-processing facilities (11.5%), were processed following ISO method and colonies which were supposed to belong to the Enterobacteriaceae family were also identified by MALDI-TOF.
Out of 248 isolates analysed, 92.7% were classified as microorganisms belonging to the Enterobacteriaceae family according to ISO rules. In contrast, MALDI-TOF analysis distributed the isolates among Enterobacteriaceae family (39.1%) and three additional bacterial families: Erwinaceae (29.6%), Yersiniaceae (28.3%) and Hafniaceae (3.0%).
These findings suggest that exclusive reliance on ISO 21528-2:2017 may lead to a substantial taxonomic overestimation of Enterobacteriaceae counts, potentially exceeding 50%. Given the ongoing lack of consensus on the taxonomic definition of Enterobacteriaceae and the inclusion of both harmless environmental species, human, plant and animal pathogens and also food spoilage within related families, further studies are needed to reassess the actual meaning of Enterobacteriaceae as hygiene indicators in the several food categories. In some cases, isolates phenotypically attributable to Enterobacteriaceae, and therefore interpreted as indicators of possible faecal contamination in food, may in fact represent microorganisms of environmental origin and *vice versa*.

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Quantifying *E. coli* in Bivalve Molluscs: A Comparative Performance Evaluation of MPN and Pour-Plate Techniques

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Bivalve molluscan shellfish (BMS) can bioaccumulate pathogens from faecally contaminated waters, posing significant public health risks via raw consumption. *Escherichia coli* remains the primary indicator for regulatory classification. Currently, the Most Probable Number (MPN) technique is the reference method; while sensitive and capable of recovering sublethally stressed cells, it is time-consuming, possesses limited statistical precision, and is characterized by inherent variability. Conversely, classical plate-count methods offer improved reproducibility and shorter turnaround times but may underestimate low-level contamination. This study evaluates the comparative advantages and limitations of these approaches.

To assess the trueness and accuracy of the pour-plate method, a comparative analysis was conducted between the reference MPN method (ISO 16649-3:2015) and the alternative plate-count method, ISO 16649-1:2018. A total of 57 naturally contaminated BMS were analysed. Additionally, sterile water samples were artificially contaminated with *E. coli* ATCC 21922 at varying concentrations (2.5 to 6.0 Log CFU/100 mL).

In the relative trueness assessment using artificially contaminated water, individual sample differences were visualized via Bland-Altman plots. Analysis showed a mean bias of -0.07013±0.2804. All data points remained within the 95% confidence limits (CLs) of agreement (-0.6197 to 0.4794). The relative trueness of the alternative method was deemed satisfactory, as no outliers exceeded the predefined acceptability limits.

For naturally contaminated BMS, the Bland-Altman plot revealed that only two out of 57 samples exhibited differences exceeding the CLs (-1.058 to 0.8848), thereby supporting the validity of the alternative method. Specifically, results obtained from *Mytilus galloprovincialis* confirmed the suitability of the pour-plate technique, with one out of 16 samples falling outside the CLs (-0.8327 to 0.6609).

The accuracy of the method was evaluated using the AP Calculation Tool (MCS) on data from artificially contaminated samples. In accordance with validation standards, the alternative method is considered equivalent to the reference method if the upper and lower β-expectation tolerance intervals fall within the established Acceptability Limits (AL). The findings demonstrated a satisfactory accuracy profile, as all values complied with the required AL.

This study validates the pour plate method as a reliable alternative to the reference MPN method for BMS, with statistical analysis confirming both trueness and precision. The tendency of MPN to yield higher estimates aligns with the Gronewold and Wolpert (2008) model [1], which attributes such discrepancies method's probabilistic basis rather than methodological bias. Consequently, the pour plate method provides a robust, reproducible, and time-efficient approach for microbiological monitoring without compromising public health safety.

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Risk assessment of *Staphylococcus aureus* in ripened artisanal goat cheeses

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The microbiological safety of dairy products manufactured from raw ovine and caprine milk of autochthonous breeds in the Basilicata region was evaluated, with a specific focus on *Staphylococcus aureus*. This pathogen represents a major public health concern due to its potential to produce heat-stable staphylococcal enterotoxins responsible for foodborne intoxication [1]. Although ripening processes in raw milk cheeses can inhibit microbial proliferation, their efficacy is strictly dependent on the initial microbial quality of the milk and the conditions during the early stages of processing. To this end, milk and cheese samples from four producers (AR, TN, PP, CL) were analysed for coagulase-positive staphylococci (CPS) load according to ISO 6888-1:2021. Two ripening methods, traditional (TR; 26 °C, 50% RH) and experimental (SP; 11 °C, 78% RH) using a Stagionello® ripening chamber [2], were compared. A representative number of strains (fifty per plate) were identified using MALDI-TOF mass spectrometry (MALDI Biotyper® Sirius, Bruker Daltonics).
The mean microbial concentration in milk was 4.43±0.53 Log (cfu/g), with *S. aureus* accounting for approximately 50% of the isolates. Following cheesemaking, CPS levels generally increased by 0.50±1.30 Log (cfu/g) in 1-day-old cheese. This wide standard deviation is primarily attributed to a single case (Producer AR, experimental ripening) where the load was 2 Log (cfu/g) lower than milk, suggesting potential thermal treatment of the milk prior to transformation. Regarding the impact of the ripening systems, CPS levels in both fresh and matured cheeses consistently exceeded the microbiological limit of established by Regulation (EC) No 2073/2005 [3] for raw milk cheeses. It is evident that during maturation, intrinsic and extrinsic factors failed to inhibit bacterial survival; conversely, the ripening period appeared to facilitate bacterial replication, with concentrations increasing by more than three logarithms, particularly under experimental conditions. In these cases, three out of four cheeses exceeded the maximum regulatory threshold (10^5 cfu/g), except for sample TN-SP which showed concentrations between minimum (10^4 cfu/g) and maximum limits. Furthermore, MALDI-TOF identification confirmed a high prevalence of *S. aureus* strains (relative abundance of approximately 95%), consistent with the raw milk data. Given that CPS levels exceeding necessitate testing for staphylococcal enterotoxins under Regulation (EC) No 2073/2005. Staphylococcal enterotoxins A–E (RIDASCREEN®) in food samples were <LOD (0.375 ng/g). The results indicate that ripening cannot mitigate the safety risk. Prevention during the milking stage and strict hygiene at the source are essential, as these toxins remain stable during both thermal treatments and maturation processes.

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Italian Beef is not a Source of Potentially Toxic Elements for General Population: Updated Evidence

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Chronic dietary exposure to potentially toxic elements (PTE) remains a major food safety concern due to their persistence, bioaccumulation, and potential to induce carcinogenic and non-carcinogenic effects [1]. In this context, meat can act as a vector for heavy metals and metalloids. In Italy, comprehensive occurrence data for bovine meat covering a broad spectrum of PTE—including emerging contaminants such as rare earth elements (REEs) whose environmental background levels are increasing due to expanding technological applications—and probabilistic dietary exposure assessments across population age classes are still limited, even though such data are essential to support accurate risk assessment. The present study aimed to determine the occurrence of 12 metals and metalloids (Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sn, U, Zn) and 14 REEs in Italian bovine meat and to perform for the first time probabilistic dietary exposure assessment and risk characterization across different population age groups.

A total of 144 diaphragm muscles were collected at slaughter from cattle reared in central-northern Italy (Piedmont, Veneto, and Tuscany). Total Hg was quantified by atomic absorption spectroscopy, while the remaining elements by inductively coupled plasma–mass spectrometry using internally validated methods. Chronic exposure through beef consumption was estimated for Italian infants, toddlers, children, adolescents, adults, and the elderly using advanced probabilistic methods, and risk was characterized by comparison with internationally established toxicological references [2, 3].

	Infants		Toddlers		Children		Adolescents		Adults		Elderly	
PTE	P50	P95	P50	P95	P50	P95	P50	P95	P50	P95	P50	P95
Zn	11%	57%	18%	69%	24%	81%	16%	61%	11%	39%	8%	30%
Fe	3%	16%	5%	20%	7%	23%	5%	17%	3%	11%	2%	9%
*i*As	43	6	26	5	20	4	30	5	43	8	62	11

Figure 1 - Contributions (%) to the provisional tolerable daily intakes of Fe and Zn, and margins of exposure (dimensionless) for inorganic As (*i*As), from bovine meat consumption across six age groups of the Italian population, estimated by Monte Carlo simulations (P50: median exposure level; P95: 95th percentile exposure level).

Zn, Fe, and Cu were the most abundant elements (1.1–58.4 mg/kg), whereas most REEs and U occurred at ultra-trace levels (0.0012–0.0738 mg/kg). Pb and Cd concentrations were consistently below European maximum levels [4], and no regulatory exceedances were observed. Total REE exposure was extremely low across all age groups (<0.008 µg/kg bw/day), and exposure to Al, Cd, Cr, Cu, Hg, Ni, Pb, Sn, and U through beef consumption was markedly below the corresponding toxicological references, indicating a low level of health concern. Conversely, in highly exposed children, beef contributed to 81% and 23% of the toxicological references of Zn, and Fe (Figure 1). The most concerning situation was observed for inorganic As, conservatively assumed to account for 100% of total measured As, as high-consumption scenarios resulted in margins of exposure ≤10 across age groups (Figure 1).

In conclusion, beef consumption does not represent a risk for the general population with respect to the intake of the investigated PTE. Nevertheless, in high consumers, exposure to Zn, Fe, and inorganic As may be significant, underscoring the need for integrated strategies combining dietary guidance and contamination reduction along the food chain to safeguard vulnerable populations.

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Blood-Based Biomarkers of Frailty in Aging Dogs: Preliminary Results from the OLD DOG Longitudinal Study

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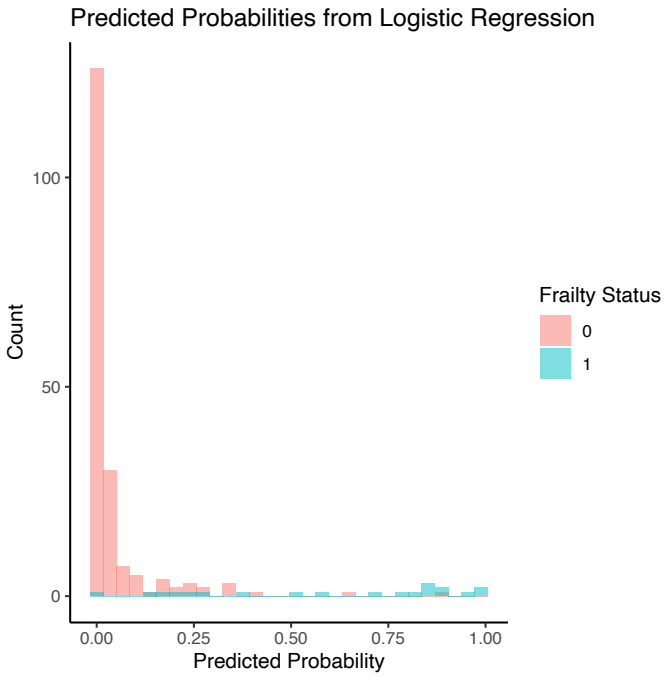


Figure 1 - Distribution of predicted probabilities of frailty based on the seven selected blood parameters in frail (red) and non-frail (blue) dogs.

Aging in companion dogs is an expanding field of research with increasing relevance for both translational geroscience and veterinary clinical practice. We present preliminary findings from the OLD DOG project, an ongoing longitudinal observational study conducted at the Veterinary Teaching Hospital of the University of Padova and aimed at identifying biomarkers of aging in dogs. To date, 209 privately owned dogs aged over five years, with no evidence of acute disease at enrollment, have been recruited at baseline. Dogs are evaluated every six months through standardized clinical examinations, owner-completed questionnaires, physical performance tests, and comprehensive blood analyses. Five follow-up visits have been completed to date, providing up to two years of longitudinal data, and a dedicated biobank has been established.

At the first assessment, frailty status was determined using a previously validated canine Frailty Index [1]. Because the FI is largely based on the accumulation of health deficits and is therefore only partially reversible, the identification of specific biological markers capable of detecting dogs at risk before overt frailty develops would be highly valuable. With this objective, we investigated whether frail and non-frail dogs could be discriminated using only routinely available blood parameters.

Thirty-one standard blood variables were examined. To identify the most informative biomarkers, we applied Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression, a penalized supervised method that performs variable selection while reducing overfitting. Model discrimination was evaluated using receiver operating characteristic (ROC) curve analysis and the area under the curve (AUC).

Seven routinely measured blood parameters demonstrated strong discriminatory ability between frail and non-frail dogs, achieving an AUC of 0.95. These included erythrocyte-related indices (RBC count, CHCM, RDW), potentially reflecting low-grade chronic inflammation; markers associated with renal function and/or hydration status (azotemia, chloride, potassium); and cholesterol levels.

Overall, these findings suggest that systemic inflammation, metabolic dysregulation, and renal or hydration-related alterations are associated with frailty in dogs. Identification of frailty-associated signatures within standard laboratory testing may facilitate earlier detection of at-risk individuals and support more personalized management strategies in aging canine populations. Ongoing longitudinal analyses will extend this approach to identify early predictors of incident frailty and to further clarify its biological progression.

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Modernizing the Dairy Food Chain: A Data-Driven Innovation for Smart Farming

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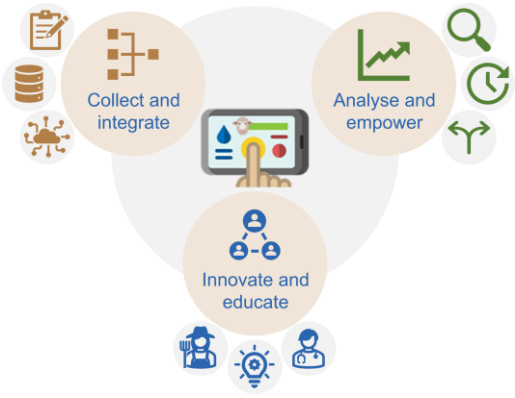


Figure 1- APPàre Approach

Introduction: While data is the currency for EU food innovation, many extensive grazing systems still lack digital analysis. The APPàre project [1][2] addresses this gap in Sardinia's dairy sheep sector by investing in innovative technologies. By enhancing digital skills, it bridges the divide between actual and potential productivity. APPàre provides decision-support tools that transform information into actionable insights, modernizing traditional livestock systems.

Methods and results: The APPàre platform is a digital tool for livestock data collection, sharing, and analysis at farm and regional scales. Initially developed through a collaborative process with veterinarians, agronomists and health partners [3], the project evolved through cascade calls, enabling farms and cheese producers to manage operations while providing feedback to identify risks and weaknesses within the production chain. The platform seamlessly integrates data from external national and regional databases [3][4][5], with real-time data from sensors (e.g., bolus readers, milk meters, weather conditions and forecasts) and with manual entries concerning animal feeding and production, medical controls, and field management. Users can monitor and manage the livestock, forage cultivation and agronomic techniques through a specialized dashboard. Furthermore, they can monitor milk quality and compare performance against regulatory alert thresholds, and against collective benchmarks for farmers or internal company benchmarks for cheese producers. The APPàre mobile app enhances efficiency by enabling rapid on-field data entry for medical checks, animal census operations, sheep status and group changes, and quick individual or batch registrations. Its modular architecture ensures compatibility with various bolus readers already owned by operators, allowing for easy expansion and integration.

Conclusions: The APPàre project highlights that a robust data-driven approach is essential for overcoming information isolation within the livestock sector. By fostering data interoperability of external databases [3][4][5], the platform provides a unified health and milk quality record that enhances epidemiological insights and supports timely decision-making. A key lesson learned is that technological innovation acts as a true catalyst only when built upon a shared vision within the industry, which subsequently unlocks broader engineering applications. Moreover, the transition to such systems requires significant education and training to ensure users can effectively navigate automated decision-making processes. Success depends on maintaining a careful balance between familiar traditional tools and new technological advancements, allowing for a digital evolution that aligns with real-world farming needs. Overall, APPàre represents a practical digital solution that enhances data interoperability, supports timely decision-making, and strengthens herd health monitoring.

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Monitoring Lambing Behavioral Changes in Ewes Using Tri-Axial Accelerometry

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Abstract

Early detection of parturition in sheep is essential for improving neonatal survival, optimizing labor management, and enhancing animal welfare in semi-intensive and extensive farming systems. Continuous visual monitoring, however, is labor-intensive and difficult to sustain. This study evaluates the feasibility of using collar-mounted tri-axial accelerometer sensors (ActiGraph) combined with machine learning and a cloud-based intelligent application to detect, monitor, and potentially predict lambing in pregnant ewes [1].

The pilot study was conducted between December 2025 and January 2026 at a semi-intensive farm in Perfugas (Sassari, Italy). Eight pregnant Black Dorper ewes were equipped, seven days before the expected lambing date, with lightweight ActiGraph sensors recording tri-axial acceleration (X, Y, Z) at 30 Hz. Collar placement followed validated methodologies in sheep behavior studies to ensure reliable motion detection while minimizing stress. Tri-axial accelerometers have been widely validated for automated behavior classification in ruminants, enabling detection of posture transitions, activity changes, and biologically meaningful behavioral states from raw acceleration signals [2].

Each lambing event was simultaneously recorded using two 24/7 video cameras to ensure full paddock coverage. Video data were downloaded three times per week and synchronized with accelerometer signals through timestamp alignment, enabling accurate behavioral validation.

Preliminary results reveal distinct alterations in activity patterns during the days and hours preceding parturition. Increased restlessness, more frequent posture transitions were observed. These changes were quantified using acceleration-derived metrics such as vector magnitude and temporal motion variability and were validated through synchronized video annotation.

Beyond feasibility assessment, this work introduces an end-to-end intelligent monitoring application. Raw sensor data are automatically uploaded to a cloud infrastructure, preprocessed, and stored in a time-series database. Machine learning models analyze temporal patterns to generate early-warning predictions of lambing onset. Results are presented through an interactive web dashboard that enables monitoring of individual animals and historical behavioral trends [3].

To improve usability for farmers and veterinarians, the system integrates a Large Language Model (LLM)-based chatbot interface. The chatbot allows natural-language queries, retrieves filtered data, and generates grounded, data-driven summaries.

This pilot trial demonstrates the feasibility of accelerometer-based lambing detection and establishes the foundation for scalable, AI-driven reproductive monitoring systems in precision livestock farming. Future work will expand the dataset and refine predictive models to improve early-warning accuracy and generalizability.

Acknowledgment

This work has been developed within the framework of the project *e.INS – Ecosystem of Innovation for Next Generation Sardinia* (cod. ECS00000038), funded by the Italian Ministry for Research and Education (MUR) under the National Recovery and Resilience Plan (NRRP) – MISSION 4 COMPONENT 2, *From Research to Business*, INVESTMENT 1.5, *Creation and Strengthening of Ecosystems of Innovation* and construction of *Territorial R&D Leaders*.

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Assessing welfare in dairy cows during hoof trimming through infrared thermography

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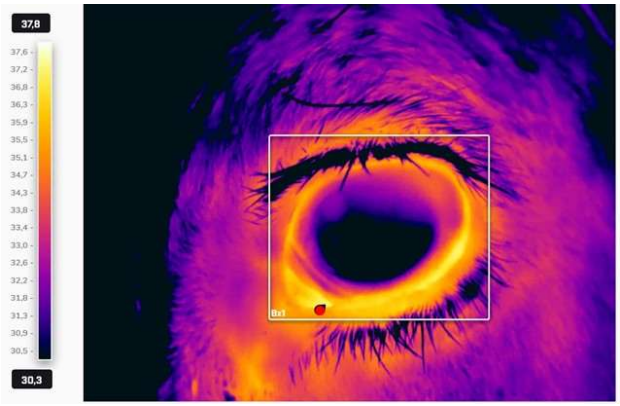


Figure 1 - Thermographic image of the left lacrimal caruncle of a cow with colour scale.

Hoof trimming, performed both to prevent and treat painful hoof disorders, can induce stress in dairy cattle due to handling and restraint [1]. Infrared thermography (IRT), a non-invasive technique for measuring surface body temperature, has been applied to the lacrimal caruncle, a region sensitive to stress responses during routine management and painful procedures [2, 3].

This study aimed to investigate alterations in eye temperature, assessed through infrared thermography, in response to routine hoof trimming, and to determine whether these changes differed according to the specific indication for trimming.

Fifty-one Holstein dairy cows of different parity and age were included in the study. Animals were selected by farm staff and moved to dedicated pens approximately one hour before hoof trimming. The procedure was performed by an experienced operator using a hydraulic trimming chute. Hoof trimming was carried out for three reasons: functional hoof trimming (FU; n = 16 cows), treatment of abscess or ulcer (AU; n = 20 cows) or treatment of dermatitis or phlegmon (DF; n = 15 cows). Three infrared thermographic images of both eyes were collected while the cow was restrained in the chute at two time points: before trimming (PRE) and after trimming (POST). Mean left and right eye temperatures were analysed using a repeated-measures ANOVA, with time (PRE vs POST) as the within-subject factor and trimming reason as the between-subjects factor.

Mean left and right eye temperatures showed minimal changes from PRE to POST in all trimming groups (FU, left: 38.15 ± 0.48 °C vs 38.17 ± 0.37 °C, right: 38.11 ± 0.42 °C vs 38.21 ± 0.37 °C; AU, left: 38.11 ± 0.45 °C vs 38.17 ± 0.46 °C; right: 38.13 ± 0.36 °C vs 38.14 ± 0.64 °C; DF, left: 38.07 ± 0.44 °C vs 38.06 ± 0.48 °C; right: 38.03 ± 0.33 °C vs 38.02 ± 0.53 °C). Repeated-measures ANOVA showed no significant effects of time, trimming reason, or their interaction on eye temperatures.

These results suggest that infrared thermography of the eye was not sensitive enough to detect changes associated with the different trimming procedures or treatments. Although the three groups may have experienced varying levels of stress related to pain or the duration spent in the chute, these differences were not reflected in eye temperature. This highlights a potential limitation of eye thermography in capturing short-term stress responses in cows under these conditions.

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A Design of Digital Twin for Smart Dairy Farming: A Systematic Review

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Digital Twin (DT) technology is increasingly recognized as a transformative paradigm for smart dairy farming, enabling the integration of real time sensor data with dynamic virtual models to support monitoring, prediction, and decision making. In dairy production systems, DT frameworks integrate data from automated milking systems, environmental sensors, computer vision, and wearable devices to model animal identification, behaviour, health status, and milk yield performance.

This review critically analyses 17 studies (from the last 5 years) to identify current trends, application domains, sustainability implications, and technical challenges in DT oriented dairy systems. The reviewed applications were categorized into animal identification and tracking (n=7), animal behaviour monitoring (n=4), animal health management (n=6), and milk yield and quality prediction (n=2), with some studies spanning multiple categories. Among these, animal health applications demonstrated the highest level of data heterogeneity and system complexity, showing the closest alignment with true DT concepts [1]. A clear technological trend toward computer vision was observed: 12 out of 17 studies employed RGB, RGB-D, or thermal cameras, reflecting a shift toward vision based monitoring and increasing adoption of deep learning models [2]. However, major challenges were reported, including occlusion in vision based systems [3], the lack of standardized datasets, extensive manual labelling requirements, and data interoperability issues.

Overall, the findings indicate strong progress toward precision livestock farming and animal welfare optimization. This review outlines the necessary technological and architectural steps required to achieve fully synchronized, farm to virtual DT systems capable of continuous data exchange and enhanced resource efficiency. Future research directions emphasize standardized frameworks, multimodal data fusion, and integration of biological modelling with AI driven analytics to enable scalable and sustainable DT ecosystems in next generation dairy farming.

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Reading between the lines: an algorithm for electroencephalogram (EEG) analysis in horses

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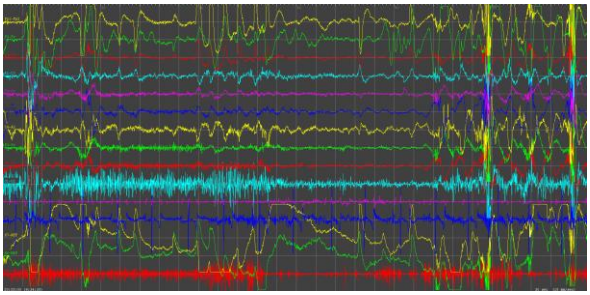


Figure 1 - Detail of a 30-second epoch illustrating wave patterns consistent with wakefulness.

Electroencephalography (EEG) is a standard method for sleep studies [1, 2]. However, although widely used in human medicine, its application to sleep analysis in veterinary medicine remains limited. One major concern of EEG is the time required for the analysis. In human sleep research, several algorithms have been developed to improve scoring efficiency [3, 4]. Therefore, the objective of this study was to analyze equine EEG recordings and develop an algorithm for sleep staging in the horse. For this purpose, a healthy 8-year-old mare underwent continuous EEG recording for 18 hours. Electrode placement followed a protocol described previously in the literature [5]. Surface electrodes were positioned at 19 predetermined sites on the equine head, including two electrooculographic (EOG) electrodes. One electromyography (EMG) electrode, the only needle electrode used, was placed in the right trapezius muscle. Recordings were obtained using a BW Mini EEG system (Neurovirtual). Data acquisition was performed in a stall, with the device secured to the halter to allow the horse to move freely. EEG, EOG, and EMG signals were acquired at 300 Hz and down sampled to 100 Hz, preserving the spectral content of interest while reducing computational burden. To minimize the influence of pre-sleep wakefulness, sensor-settling effects, and transition-related artifacts, the first 30 min of recording were excluded. The remaining data were segmented into 30s epochs, and spectral features were extracted using the Welch method. Relative sigma and delta power were computed by normalizing EEG sigma (12–15 Hz) and delta (0.5–4 Hz) power to total EEG power in the 0.5–35 Hz range, averaged across central and frontal derivations. Band-limited power was also computed for EOG and EMG signals and subsequently log-transformed to improve the robustness of percentile-based thresholding. All preprocessing, feature extraction, and rule-based staging procedures were implemented in Python. Sleep stages were assigned using physiologically grounded decision rules and recording specific percentile thresholds derived from EMG, EOG, and EEG spectral features, followed by temporal smoothing. In the 20% validation set (n = 406 epochs), the method achieved an overall accuracy of 82.0%, with class-wise recall of 68.7% for N1, 70.8% for N2, 14.3% for N3, 84.6% for REM, and 88.5% for wakefulness. The low recall observed for N3 likely reflects the limited representation of this stage in the validation set. Although these findings remain preliminary, as they derive from a single-horse recording and therefore do not provide clinical validation, they support the feasibility of this approach and encourage further evaluation in a larger equine cohort.

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In-field Validation of an IoT-Based System for Monitoring Ammonia and Fine Particulate Matter from Livestock Buildings

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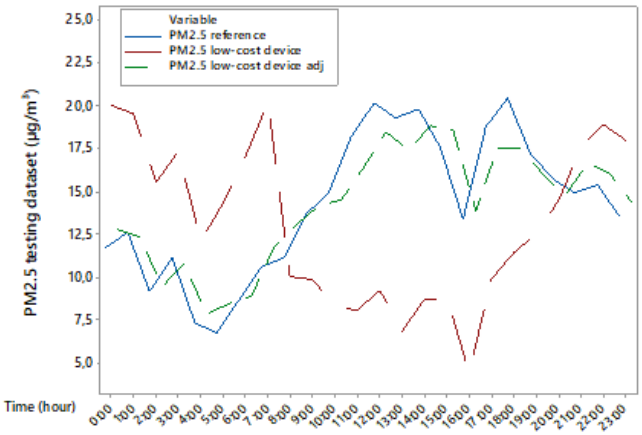


Figure 1 - $PM_{2.5}$ concentration daily trends related to the reference device, low-cost device and low-cost device adjusted, for the testing dataset.

The livestock sector represents one of the predominant sources of atmospheric emissions of both gaseous and particulate pollutants, notably ammonia (NH_3) and fine particulate matter ($PM_{2.5}$). Continuous and reliable monitoring of these pollutants is essential for environmental assessment and for the development of effective mitigation strategies. However, the widespread adoption of monitoring instruments is often limited due to the high costs of both devices and operational maintenance.

In this study, the performance of low-cost sensors integrated into an Internet of Things (IoT) monitoring system [1] for the simultaneous monitoring of NH_3 and $PM_{2.5}$ concentrations and environmental variables was analysed in a livestock building. The experimental activity was carried out in a dairy barn located in Mediterranean area. Data acquired by the low-cost NH_3 and $PM_{2.5}$ sensors were validated against high-precision reference instruments, including an INNOVA photoacoustic analyser for NH_3 concentrations (Lumasense Technology A/S, Denmark), and an MP101M reference monitor for $PM_{2.5}$ (ENVEA, France). The sampling points of the IoT system were selected to align withthe inlet height of the reference instruments to ensure measurements comparability at the same air sampling points and minimising potential systematic errors. Data analysis was carried out to assess the reliability of the low-cost IoT system by using measurements acquired by the reference devices as a benchmark.

The IoT system demonstrated a reasonable ability to monitor average $PM_{2.5}$ and NH_3 concentrations in a livestock environment. The results highlighted that the performance of the IoT system was influenced by environmental variables, particularly air temperature and relative humidity. Therefore, correction models based on climatic parameters were developed to achieve reliability comparable to reference devices. In detail, a multiple regression analysis provided two equations to correct $PM_{2.5}$ concentrations by using the thresholds of 75% and 14.5 °C, for air relative humidity and air temperature, respectively. Based on these corrections of $PM_{2.5}$ concentrations, the correlation indices between $PM_{2.5}$ concentration of the reference device and adjusted $PM_{2.5}$ concentrations of the Io-T system was 0.79. For NH3 concentrations, the correction was based on multiple regression analysis, producing a correlation index of 0.64 between NH_3 acquired by reference and adjusted NH_3 concentrations for the low-cost device.

After applying the proposed correction models, the adjusted sensor data showed good agreement with reference measurements (Fig.1), demonstrating that the analysed low-cost devices are suitable for monitoring mean daily NH_3 and $PM_{2.5}$ concentrations within livestock houses. Although the correction models were developed by using data collected in a dairy barn, the proposed methodology can be applied to similar livestock environments characterised by comparable emission sources and microclimatic conditions.

This study demonstrated the potential of low-cost, IoT-enabled monitoring systems as effective tools for distributed air quality assessment in animal production systems. Due to their affordability, portability, and ease of installation, these sensors can be deployed at multiple locations within a barn or relocated among different facilities, enabling flexible and spatially resolved monitoring. Such approaches can support data-driven emission mitigation strategies, improved barn management, and the broader digital transformation of sustainable livestock farming.

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Quantifying NH_3 Emissions in Naturally Ventilated Dairy Barns: A Decade of Methodological Advancements and Challenges

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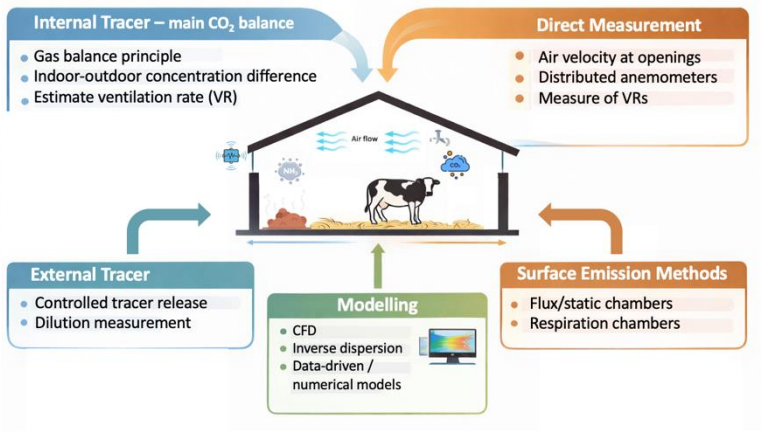


Figure 1 - Overview of measurement strategies for NH_3 emissions in naturally ventilated dairy barns.

The quantification of ammonia (NH_3) emissions from naturally ventilated dairy barns (NVBs) represents a complex measurement system challenge, where airflow variability, sensor configuration, and climatic conditions interact to determine overall uncertainty [1-2]. Although several studies have been conducted in recent years on dairy farms, monitoring emissions remains challenging as there is no universally validated Standard Reference Method (SRM), which limits comparability and scalability [3-4]. Moreover, there is a growing need for long-term monitoring systems capable of quantifying emissions accounting for seasonal variability and routine husbandry practices.

This study presents an integrated technical and bibliometric analysis of measurement strategies adopted in NVBs over the last decade, focusing on methodological evolution, uncertainty propagation, and climatic representativeness.

A geographical analysis of 81 peer-reviewed studies reveals a strong concentration of research activity in Northern and Central Europe, while Mediterranean systems (Italy, Spain, Portugal combined <10%) and warm-climate regions remain underrepresented. Most represented protocols are optimized for ridge-ventilated cubicle barns under temperate climates and may not be directly transferable to Mediterranean barns characterized by large lateral openings, mixed wind–buoyancy regimes, high solar loads, and weaker winter thermal gradients.

Temporal analysis of methodological adoption shows clear consolidation around two dominant approaches: CO_2 mass balance and direct airflow measurement using distributed anemometers. The first approach represents 37% of applications, followed by direct airflow measurements with anemometers (21%). Modelling approaches are showing increasing adoption, including computational fluid dynamics (10%), wind tunnel studies (6%), and numerical or semi-mechanistic models (~5%), which are demonstrating their value as supporting tools. The CO_2 balance method maintains stable application across years due to scalability and operational simplicity, whereas direct airflow measurement with anemometers promises to be SMR for validation of more feasible protocols and equivalent methods. Emission factors (EFs) reported in the dataset range between 0 and 2.4 g h^{-1} LU^{-1}, with coefficients of variation approaching 50%, and part of this variability may be attributable to measurement configuration.

Developing robust and scalable monitoring frameworks therefore requires structure-sensitive and climate-adaptable measurement protocols that explicitly account for specific external climate conditions, barn geometry, ventilation regime, and management practices, as these factors fundamentally influence airflow dynamics, pollutant dispersion, and overall measurement uncertainty.

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Low-cost drone system for monitoring ammonia and GHG emissions in dairy barns: a case study in southern Italy

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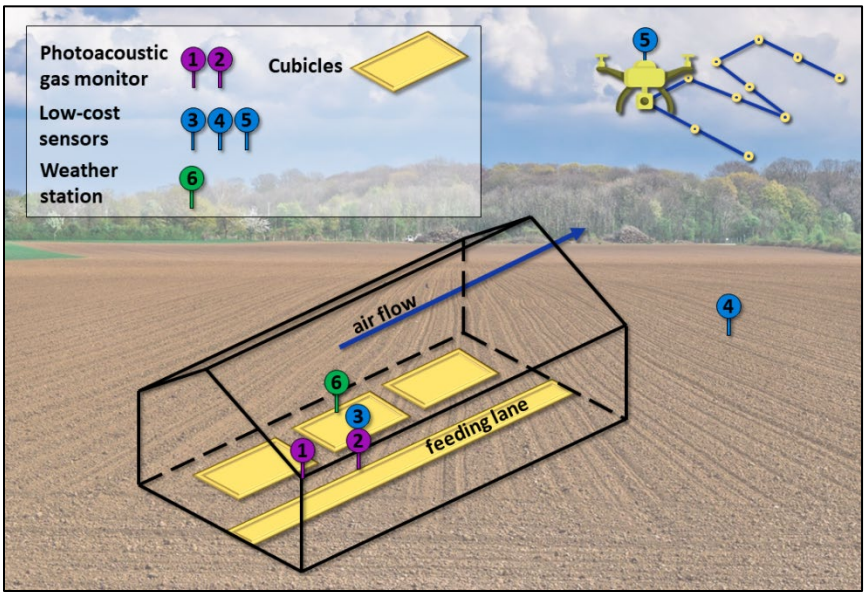


Figure 1 – Experimental setup of the trial for validating a prototype low-cost sensing system for ground and aerial gas measurement.

Bottom-up approaches for estimating greenhouse gas and ammonia (NH_3) emissions from livestock farms require gas concentration measurements at the farm level. A major limit to their applicability across diverse farming and environmental contexts is the reliance on high-cost standard instruments, with several operational limitations [1]. This study aimed to validate a prototype low-cost sensing system for ground and aerial measurements, designed to monitor concentrations and estimate NH_3 and CO_2 emissions of a dairy barn in Mediterranean area. The experiment was conducted over one week during the hot season at a facility housing 50 lactating cows (Fig. 1). The system, previously described in [2], consisted of two prototype devices equipped with low-cost sensors to monitor gas concentrations and environmental parameters. One device was installed at a fixed ground station downwind of the main airflow (Fig.1, point 4), while the second was mounted on a mobile drone (point 5). Wind speed and direction were measured using a 2D anemometer placed inside the barn (point 6). Additionally, measurements from the low-cost devices (point 3) were acquired simultaneously with those from an infrared photoacoustic gas monitor (point 2), used as reference instrument, to derive correction equations for both NH_3 and CO_2 concentrations. Finally, the corrected measurements from the low-cost devices were used to estimate NH_3 and CO_2 emissions during and after specific barn operation (i.e., slurry removal and feed delivery) using the WindTrax dispersion model. Estimated emissions were compared using the Mann-Whitney U test.

NH_3 emissions during slurry removal (0.00037 g m^{-2} s^{-1}) were significantly higher ($p < 0.001$) compared to those at the end of cleaning procedures (0.00020 g m^{-2} s^{-1}), while the opposite trend ($p < 0.001$) was observed when comparing NH_3 emissions during (0.00025 g m^{-2} s^{-1}) and after (0.00034 g m^{-2} s^{-1}) feed distribution. Regarding CO_2 emissions, higher values ($p < 0.001$) were estimated during cleaning operations (0.1386 g m^{-2} s^{-1}) than after slurry removal (0.0487 g m^{-2} s^{-1}), while no significant differences were found ($p = 0.379$) between emissions during (0.0340 g m^{-2} s^{-1}) and after (0.0392 g m^{-2} s^{-1}) feed distribution.

Overall, the system showed positive performance in measuring gas concentrations after correction against a reference instrument and in capturing emissions fluctuations across diverse phases of barn management. Developing automated, low-cost systems thus represents a promising solution for both tailoring emission factors to local conditions and providing immediate feedback to farmers, enabling them to adopt timely mitigation measures.

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High-frequency environmental measurements for data-driven livestock management

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Environmental conditions inside livestock buildings strongly influence animal health, welfare and productivity, while also affecting emissions and the environmental footprint of livestock production. Despite this, environmental parameters in commercial farms are often monitored only sporadically, limiting the ability to detect harmful microclimatic events or to understand the relationship between environmental dynamics and animal health outcomes.

This work presents the development and field deployment of an AI-IoT environmental monitoring system designed for continuous measurement of key parameters in livestock facilities. The system integrates distributed sensor nodes capable of measuring temperature, relative humidity, carbon dioxide (NDIR), ammonia and hydrogen sulphide (electrochemical), methane (NDIR), particulate matter (optical), light intensity and peak noise levels (membrane microphone). Additional parameters can be monitored through optional sensor modules depending on the application. Each sensor node communicates with a local gateway, which transmits data to a cloud platform via GSM or Wi-Fi connectivity. The system is powered through mains supply and supports up to eight sensor modules per gateway, enabling flexible deployment within livestock buildings.

Measurements are collected at high temporal resolution: sensors sample environmental variables every 30 seconds and compute averaged values over 20-minute intervals (±5 minutes), resulting in approximately three validated measurements per hour transmitted in near real time. This sampling strategy was selected to capture short-term environmental fluctuations (e.g. ventilation cycles or emission peaks) while ensuring robust and stable data for farm-level decision-making through temporal aggregation.

The system has been deployed in more than 40 commercial livestock farms with over 300 devices installed, generating continuous datasets over typical monitoring periods of approximately one year. Periodic recalibration (every 12 months) is implemented to maintain measurement reliability under harsh environmental conditions.

Field observations demonstrate the practical value of continuous environmental monitoring for management decisions. In a sow farm in Italy hosting approximately 900–1000 piglets, the system detected recurrent peaks of hydrogen sulphide that had previously gone unnoticed. Following management adjustments informed by these measurements, overall piglet mortality decreased from 15% to 11%. In a second case study in a fattening pig farm in northern Italy (590 pigs), continuous monitoring of ammonia concentrations supported improvements in ventilation and barn management, which were associated with improved air quality and a 20% reduction in antibiotic treatments for respiratory diseases over one year.

Some limitations should be acknowledged. Measurement variability can be influenced by sensor placement within the barn, although this is mitigated through defined installation guidelines. In addition, periodic calibration is required due to the harsh operating environment, and the system is not intended to replace reference-grade instrumentation. Connectivity constraints in remote rural areas may also affect data transmission performance.

Future developments will focus on refining predictive models derived from environmental data and expanding sensing capabilities to address additional challenges identified by livestock producers.

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An IoT-Based Multi-Gas Monitoring Platform for Real-Time Air Quality Assessment in Livestock Buildings

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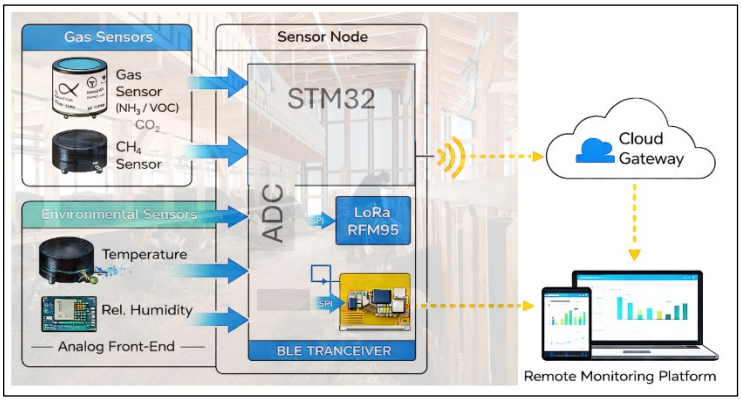


Figure 1 – Schematic diagram of the wireless multi-gas sensing platform for real time air quality monitoring in livestock building.

Air quality inside closed livestock housing plays a crucial role in animal welfare, worker safety, and environmental sustainability. Intensive farming systems are often characterized by the accumulation of gases produced by animal respiration, manure decomposition, and feed fermentation processes. Among these, ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4), and volatile organic compounds (VOCs) are considered key indicators of environmental conditions within livestock facilities [1],[2]. Elevated concentrations of these gases may negatively affect respiratory health, animal productivity, and overall welfare, while also contributing to environmental emissions [3]. For these reasons, continuous monitoring of air quality parameters is increasingly recognized as an important tool for improving environmental management and supporting evidence-based decision-making in modern livestock farming [4, 5].

In this work, we present a wireless Internet-of-Things (IoT) sensing platform designed for real-time monitoring of environmental conditions in livestock buildings. The proposed system is based on a modular sensing node capable of integrating multiple gas sensors together with environmental sensors. The platform can host sensors for NH_3, CO_2, CH_4, and VOCs, based on different technology, as well as temperature and relative humidity sensors. The sensing node is designed to operate with low power consumption and to transmit data wirelessly to a remote infrastructure. The node supports data transmission via both Bluethoot Low Energy (BLE) and Long Range (LoRa) technology, enabling integration into distributed monitoring systems. At its core is the low power STM32WB55 microcontroller, embedding BLE capabilities enabling continuous surveillance of environmental conditions and remote access to measurements. The modular architecture allows flexible sensor configurations depending on the monitoring requirements of different livestock production systems.

To evaluate the feasibility of the proposed system, preliminary experimental measurements were carried out using a pellistor type CH_4 sensor and an electrochemical VOC sensor integrated within the sensing platform. The results demonstrate the capability of the system to detect variations in gas concentration and to transmit environmental data in real time. These initial tests confirm the suitability of the sensing architecture for continuous monitoring applications in livestock environments. The proposed platform represents a promising technological solution for veterinary environmental monitoring. By providing real-time information on barn air quality parameters, the system may support improved ventilation management, early detection of unfavorable environmental conditions, and more efficient livestock facility management. Furthermore, the deployment of distributed sensing networks based on the proposed architecture could contribute to improved animal welfare monitoring and to the reduction of environmental emissions associated with intensive livestock production.

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Evaluation of an automatic feeding system: effects on lactating sow feed intake and piglet growth

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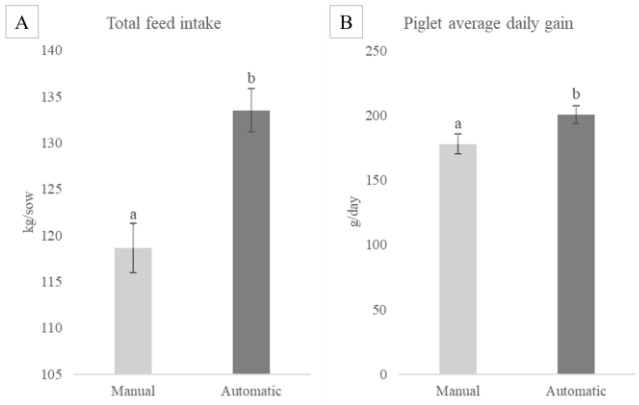


Figure 1 - (A) Total feed intake of sows during lactation and (B) piglet average daily gain (ADG) in sows fed manually or with an automatic feeding system.

Feed intake during lactation is a key determinant of sow energy balance, milk production, and piglet growth performance. Insufficient voluntary feed intake during this phase has been consistently associated with increased body reserve mobilization in sows and reduced litter growth [1]. Automatic feeding systems, which allow sows to regulate feed intake throughout the day, have been proposed as a strategy to stimulate voluntary feed intake during lactation and improve productive performance under commercial conditions [2,3]. The experiment was conducted from July to December 2025 over five weekly batches, involving a total of 70 Goland PIC sows equally allocated to two experimental treatments (35 sows per treatment). In the control group (C), sows were fed manually using a traditional mechanical system delivering 980 g of feed per meal, distributed several times per day. In the automatic feeding group (AF), sows were fed using GESTAL Solo+ (JYGA Technologies) that allowed voluntary feed intake at any time of day. At preset intervals, small amounts of feed were released into the trough to stimulate sow activity; activation of a mechanical sensor by the sow triggered delivery of a meal. A dry pelleted diet was used in both treatments. Sows were assigned to treatments according to parity, and none had previous experience with automatic feeding systems. Backfat thickness was measured at the beginning and end of lactation using both a sow caliper and ultrasonography technology (Renco). Piglets were weighed after litter equalization and prior to weaning at 25 days of age. AF group showed a significantly higher total feed intake during lactation compared with C group (133.5 vs 118.6 kg/sow; +14.9 kg, $p < 0.0001$), corresponding to an average increase of 0.71 kg/day. This increased feed intake was associated with improved litter performance, as piglets from AF group had a significantly higher average daily gain (ADG) compared with the C group (200.5 vs 177.9 g/day; +22.6 g/day, $p = 0.034$) and showed a tendency for greater litter weight at weaning (6.69 vs 6.13 kg; +0.56 kg, $p = 0.065$). No significant differences were observed between treatments in sow backfat thickness, regardless of measurement method used. In agreement with previous studies, increasing voluntary feed intake during lactation improved litter growth without negatively affecting sow body condition [1,2]. The use of automatic feeding system during lactation therefore represents an effective management strategy to enhance feed intake and piglet growth performance under commercial farming conditions [2,3].

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Precision Livestock Farming for Tail Biting Management in a Commercial Breeding Environment

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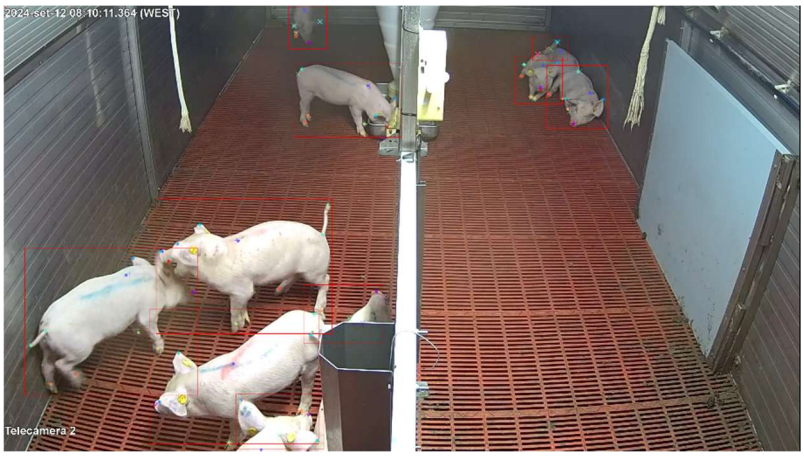


Figure 1: Sample frame of the pose estimation output highlighting a biting event and the corresponding fictitious keypoint representation.

Introduction

Tail biting (TB) remains a significant welfare and economic challenge in pig farming, particularly as the industry transitions toward undocked populations. While several risk factors are known, objective and continuous monitoring in commercial settings is often lacking. This study explores the integration of multi-parametric environmental sensors and advanced behavioral analysis tools to assess tail biting dynamics in a non-experimental, commercial breeding environment.

Methods

The study was conducted in a commercial breeding nucleus in Northern Italy, monitoring groups of Large White gilts in outdoor climate-controlled huts. To improve environmental adequacy, specialized sensor units were installed to provide continuous data on temperature, humidity, and concentrations of harmful gases such as ammonia and carbon dioxide. Simultaneously, an automated video analysis pipeline based on deep learning pose estimation was deployed to monitor social interactions. The system utilized interaction modeling techniques to identify contact between individuals, while individual identification was supported by vision-based models for ear tag recognition. Behavioral data were further compared with clinical assessments and physiological indicators of chronic stress.

Results

The integrated monitoring system provided a comprehensive overview of the relationship between housing conditions and animal behavior. Environmental sensors identified significant microclimatic fluctuations, providing a baseline to explore environmental instability as a potential contributing factor to behavioral anomalies. The behavioral analysis module successfully detected social interactions indicative of biting events, even under the structural constraints and high animal densities typical of commercial pens. By filtering raw video data through spatial and temporal constraints, the system demonstrated its capability to isolate relevant events from background activity, providing a manageable output for veterinary surveillance without the need for constant human observation.

Conclusions

The combination of sensor-based environmental monitoring and automated behavioral analysis represents a robust framework for improving animal welfare in commercial herds. These results suggest that Precision Livestock Farming (PLF) solutions can effectively support veterinarians in identifying early warning signs of tail biting and optimizing housing conditions. The study highlights the feasibility of implementing such technologies in real-world production settings, provided that environmental and structural complexities are integrated into the system's design. (Project RC 9/2022, CUP: B23C22000930001)

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Economic impact of porcine epidemic diarrhea virus in growing pigs: an integrated algorithm combining causal inference analysis and a full-budget model

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Porcine epidemic diarrhea (PED) remains a major economic issue in swine production, leading to significant financial losses [1]. Prior studies often used simplified methods that overlooked complexities in large-scale systems. This research applied the detailed Iowa State University full-budget model [2], which captures cost-performance dynamics in modern operations, combined with advanced causal inference to isolate PED true effects while controlling confounders. The aim was to assess PED real-world economic impact in wean-to-finish pigs at a large swine operation.

Data came from a major U.S. swine company's records. After cleaning and excluding lots with other diseases, 2229 marketed lots (Jan 2022–Dec 2024) were analyzed. Lots were grouped by PED status (positive/negative) and infection timing (wean or marketing break). Losses were estimated via an adapted ISU full-budget algorithm, reported as net income change and opportunity cost ($/head). Impacts were evaluated with univariate modeling (UM) and doubly robust (DR) methods using augmented inverse probability weighting, adjusting for weaning season, mass antibiotics, stocking density, pre-weaning mortality, lactation length, and sow farm PRRS condition.

Among lots, 48 had wean breaks and 267 had marketing breaks. Wean breaks linked to –$6.96 (UM) and –$4.60 (DR) net income per head, plus $4.09 (UM) and $3.08 (DR) opportunity loss (both $p < 0.05$). Performance showed +2.62 days on feed, +0.03 worse feed conversion, –5.09 lb lower carcass weight, and +3% higher mortality. Marketing breaks showed UM estimates of +$1.73 opportunity cost ($p < 0.05$) and –$2.42 net income. DR adjustment reversed this to +$2.19 opportunity loss and –$5.58 net income, with performance changes of –0.19 days on feed, +0.09 worse feed conversion, –2.94 lb lower carcass weight, and –1% lower mortality.

PED causes clear performance and economic harm in growing pigs [3]. Wean infections produced ~$1/head more loss than marketing ones, highlighting infection timing's importance. UM and DR aligned for wean breaks, but only DR correctly revealed marketing-break effects, where unadjusted estimates underestimated and reversed the true impact. These results show causal inference outperforms traditional univariate methods in observational veterinary data [4]. Integrating full-budget modeling with rigorous adjustment offers producers a precise, whole-system view of PED under field conditions, aiding better biosecurity, management, and strategic decisions.

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Piglet crushing mechanisms in temporary crating systems: insights from continuous video monitoring

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Figure 1 - Overhead camera view of the farrowing pen equipped with a temporary crate, used for continuous behavioural monitoring of sow–piglet interactions.

In response to the European Citizens' Initiative "End the Cage Age", the European Commission announced a revision of EU animal welfare legislation aimed at phasing out cages for farm animals, potentially including farrowing crates for sows. Although the legislative proposal has not yet been finalized, the transition toward housing systems without permanent confinement is widely anticipated. In this context, temporary crating systems have been proposed as a compromise solution, allowing sow mobility during most of lactation while reducing piglet crushing risk in the first days after farrowing [1]. However, piglet mortality remains a major concern, particularly in modern hyperprolific sows characterized by large litters and greater within-litter birth weight variability [2].

The aim of this study was to describe sow behaviour in the minutes preceding crushing events in systems without permanent confinement, in order to identify prevalent posture changes associated with piglet crushing and support the development of mitigation strategies. Continuous high-resolution video monitoring was implemented as a precision measurement tool.

The study was conducted on a commercial 750-sow farrow-to-wean farm in northern Italy. Sixty-eight sows (parity 1–5; mean ± SD: 2.7 ± 1.46) were randomly selected. Sows farrowed in individual pens (7.10 m²) equipped with temporary crates that were opened on average 7 days postpartum. Behaviour was continuously recorded using overhead cameras (DH-IPC-HFW3441E-SA, Dahua) mounted at 2 m height, covering the entire pen until weaning. Videoclips were extracted from approximately two minutes before each crushing event until the piglet became visible again after the sow moved. An ethogram was developed to classify fatal posture changes and sow behaviours immediately before and after the event.

A total of 106 crushing events were analysed. Thirty-five percent occurred while the crate was closed (n = 40), whereas 65% occurred after crate opening (n = 75) (P = 0.001). When crates were open, crushing was mainly associated with rolling movements (75%), whereas standing-to-lying transitions predominated when crates were closed (69%).

These results indicate that the mechanisms leading to piglet crushing differ according to the degree of sow freedom of movement. The transition toward systems without permanent confinement may therefore modify, rather than simply increase, crushing risk patterns. Continuous video monitoring proved to be a valuable precision measurement tool to identify behavioural sequences preceding crushing events and may support the development of automated systems to detect risky sow–piglet interactions in welfare-oriented farrowing systems.

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Unmasking the underdog: Advanced molecular strategies to improve piroplasm characterisation and unveil co-infections in dogs and ticks

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Canine tick-borne diseases represent a significant challenge in sub-Saharan Africa, where diagnostic limitations often hinder effective surveillance [1]. A critical scientific concern with using generic molecular diagnostic approaches targeting conserved genetic regions is the "masking effect", in which a high parasitemia of one pathogen can prevent the detection of others. In 2023, blood samples from 273 dogs of Southern Ethiopia were molecularly screened by end-point PCR using generic 18S rRNA primers that amplified a short fragment (~373 bp), revealing a high prevalence of *Hepatozoon canis* (53.8%) but a notably lower prevalence of *Babesia rossi* (3.3%) [2]. The short fragment did not allow an appropriate phylogenetic analysis necessary to evaluate the clustering of detected strains and determine taxonomic relationships.

In 2025, to enhance the understanding of the epidemiology of circulating pathogens, 301 ticks (167 adults and 134 nymphs) collected from the same dog population were evaluated using probe-based quantitative qPCR assays (targeting the 18S rRNA genes) of *H. canis, B. rossi, B. canis*, and *B. vogeli* [3, 4]. Additionally, to refine previous measurements, the positive canine blood samples (n=49) were re-evaluated, using qPCR.

The complete (~1600 bp) 18S rRNA gene fragment was amplified and sequenced from a selected number of positive samples (blood and ticks) [5]. Sequences were assembled and edited using Chromas Pro, and MEGA was used to infer phylogenetic relationships relative to reference sequences available in Genbank.

The qPCR analysis of ticks identified 84/301 samples (i.e., *Rhipicephalus* and *Haemaphysalis* genera) positive for *H. canis,* one of which was co-infected with *B. rossi* (i.e., *H. leachi*). In canine samples, probe use resulted in a higher *Babesia* detection rate; indeed, six blood samples were found to be coinfected, revealing low-level infections previously obscured by the prevalence of *H. canis*. Amplification of the complete 18S rRNA gene yielded a robust dataset for phylogenetic placement, confirming the identity of the circulating strains. *Babesia canis* and *B. vogeli* were not detected in dogs or the tick samples, indicating their absence in the study area

This study shows that molecular "masking" can be a challenge in multi-pathogen environments. By transitioning from generic short-fragment assays to species-specific probes and long-fragment PCR, a more accurate assessment of piroplasm diversity was achieved. These molecular methods are crucial for reliable molecular epidemiology of pathogens (i.e., *B. rossi* and *H. canis)* in this region. A better understanding of circulating strains will enable more accurate veterinary diagnostics.

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Molecular detection and genospecies diversity of *Borrelia* in *Ixodes ricinus* ticks from an endemic area of Northeastern Italy

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Lyme borreliosis is a common tick-borne zoonosis in Europe, primarily transmitted by *Ixodes ricinus*. It is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex, including *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii* and *B. miyamotoi* while other genospecies, such as *B. valaisiana*, *B. lusitaniae* have also been reported, though their pathogenicity remains unclear [1,2]. This study investigated the occurrence, genospecies distribution and genomic characteristics of *Borrelia* spp. in ticks collected between 2021–2025 in the Belluno province (Veneto, North-Eastern Italy), where ticks are abundant and many tick-borne zoonoses, including borreliosis, are endemic [3,4].

Ticks were collected in six municipalities through 98 dragging transects (100 m² each). All 11,588 specimens were identified as *I. ricinus*: 7,233 larvae (L), 4,498 nymphs (N), and 357 adults (A; 150 males, 207 females). Adults were analyzed individually, while larvae and nymphs were pooled (≤15 specimens per pool), yielding 1,546 samples (737 L pools, 452 N pools, 357 A).

DNA was screened for *Borrelia* spp. by real time PCR using a FAM-labeled TaqMan assay. Positive samples were subsequently characterized by two multiplex real-time PCR assays targeting six genospecies: triplex 1 (*B. miyamotoi* – Cy5; *B. afzelii* – ROX; *B. valaisiana* – FAM [included in 2025 only]) and triplex 2 (*B. burgdorferi* s.s. – FAM; *B. garinii* – Cy5; *B. lusitaniae* – JOE). Molecular characterization by Multi Locus Sequence Typing (MLST) was performed on 14 samples of Borrelia-positive ticks and 5 reference strains.

Overall *Borrelia* occurrence was 16%, highest in nymphs (42%), followed by adults (10%) and larvae (3%). Seasonal occurrence peaked in winter (February-March, 23%), followed by spring (April-June19%), autumn (September-November 11%), and summer (July-August, 9%). All genospecies were detected across life stages except *B. valaisiana*, found only in pooled nymphs.

Coinfections were mainly observed in adults, most frequently involving *B. miyamotoi* and *B. garinii*. Nymph pools often harbored multiple genospecies, and one pool contained four distinct genospecies (*B. afzelii, B. garinii, B. lusitaniae,* and *B. valaisiana*). MLST analysis identified 13 new sequence types, demonstrating marked genetic heterogeneity and no evidence of clonality between strains from environmental ticks.

In conclusion, a high diversity of *Borrelia* genospecies was observed in this endemic area, including a sample with four distinct genospecies. The highest occurrence in winter indicates no season is no-risk, suggesting climate change may influence pathogen circulation. The multiplex real-time PCR assay proved rapid and effective for epidemiological surveillance and monitoring of *Borrelia* genospecies in endemic areas.

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Molecular detection of *Echinococcus multilocularis* and cestode infections in Alpine small rodents

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Echinococcus multilocularis (Em), the causative agent of human alveolar echinococcosis, is expanding across Europe, including Alpine regions where heterogeneous ecological conditions influence parasite transmission [1]. Reliable surveillance on this zoonotic parasite requires sensitive and specific molecular tools, capable of detecting Em throughout its life cycle in both definitive and intermediate hosts [2][3]. This study applied standardized molecular methods to detect Em and characterize metacestode infections in selected small rodent communities from North-Eastern Italy, improveing eco-epidemiological understanding and surveillance sensitivity.

Sampling sites were selected based on prior epidemiological evidence of Em presence and rodent habitat suitability. Fieldwork was conducted in three Alpine areas in the province of Bolzano with different epidemiological profiles: Val Giovo (endemic for Em), Val d'Ultimo (apparently Em-free), and San Leonardo in Passiria (sporadic Em presence Em). In accordance with ethical committee approval, active trapping was carried out in August/September 2024 and July 2025 in Alto Adige and yielded 87 small rodents, mainly *Apodemus flavicollis* and *Myodes glareolus*, while passive surveillance contributed an additional 162 specimens, primarily *Arvicola amphibius* and *Apodemus sylvaticus*. Captured and passively collected individuals were subjected to necropsy. Nucleic acids were extracted from mice livers (cysts and/or parenchyma) using MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems™) on an automated Microlab STARlet workstation (Hamilton Company). Detection of Em DNA was performed using a real-time probe multiplex PCR assay targeting the mitochondrial COI gene with an internal control. All samples were further analyzed using multiplex endpoint PCR targeting mitochondrial markers, followed by sequencing, to identify other cestode species.

To date, 145 mouse livers have been analyzed using real-time multiplex PCR (46 from active trapping, 99 from passive surveillance). Only one *M. glareolus* from Val Giovo tested positive for Em. Furthermore, 24 samples were identified as positive for *Taenia taeniaeformis* (n = 19) and *Mesocestoides litteratus* (n = 5) by multiplex endpoint PCR and sequencing. These parasitic cestodes typically involve rodents as intermediate hosts and carnivores as definitive hosts.

Molecular techniques applied in this study allowed the detection of Em and other cestode infections in wildlife, and consistent with their biological and epidemiological features (e.g. Em detected only in the endemic area). However, further studies are needed to assess the cost-effectiveness of molecular-based monitoring in small rodent communities and its potential to enhance the detection of Em circulation, even at low prevalence. A better understanding of the eco-epidemiological role of different rodent species is essential to develop cost-effective, high-sensitivity surveillance strategies within a One Health framework in Alpine ecosystems.

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Integrating lactation and dry off data to predict post calving intramammary infections in dairy cows

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Antimicrobial resistance (**AMR**) represents a major threat to both human and animal health and is largely driven by excessive and inappropriate antimicrobial use (**AMU**) [1]. In dairy cattle, the greatest proportion of AMU is associated with the treatment of intramammary infections (**IMI**), with the highest risk of new infections occurring during the dry period and post calving [2, 3]. Subclinical IMI are commonly associated with elevated somatic cell count (**SCC**), which negatively affects milk quality, animal welfare, and productivity [3]. To support targeted management strategies aimed at reducing antimicrobial use and improving animal welfare, this study evaluated whether cows at risk of elevated post calving SCC or intramammary infection can be identified using machine learning applied to integrated lactation performance, on-farm sensor data and bacteriological analysis collected at dry off.

A total of 294 dairy cows were sampled at dry off, with quarter-level milk samples collected for bacteriological analysis. Lactation data included linear score, milk yield, fat and protein production from Dairy Herd Improvement (**DHI**) records, number of clinical mastitis cases, duration of lactation and dry period, milk yield at dry off, parity, teat condition score, and dry off treatment. Sensor data included rumination, activity and health status from collars and milk flow and milk production distribution from milking parlour.

Two binary outcomes were modeled: SCC ≥200,000 cells/mL at the first post-calving DHI test and infection by a major pathogen 2–8 days after calving. Four supervised learning approaches were compared: Logistic Regression, Random Forest, XGBoost, and Support Vector Machine (**SVM**). Data were divided into training (70%) and test (30%) sets using stratified sampling. Hyperparameters were optimized using repeated 5-fold cross-validation (2 repeats), selecting models based on balanced accuracy.

Our preliminary analysis showed that balanced accuracy predicting high SCC ranged from 61% to 69%, with Random Forest performing best. For predicting post calving IMI, balanced accuracy ranged from 55% to 75%, with Logistic Regression showing the highest performance.

Although predictive performance was moderate, this study indicates that the integration of sensor data with lactation and clinical records has the potential to classify cows into distinct risk groups for both post calving IMI and elevated SCC, supporting farmers and veterinarians in decision-making processes and potentially reducing antimicrobial use while improving animal welfare.

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Detection of coronet temperature changes in horses using infrared thermography during partial limb occlusion and reperfusion

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Skin temperature is determined by blood temperature and cutaneous blood flow, which is regulated primarily through sympathetic adrenergic vasoconstrictor activity. Thus, factors affecting periferic vasodilatation and vasoconstriction, such as diseases, injuries, and compression can alter infrared radiation, leading to an increased or decreased periferic body temperature. Infrared thermography (IRT) is a diagnostic imaging technique that captures infrared radiation emitted by the body and converts into thermal data [1, 2]. Recently, it has emerged in equine veterinary practice as a promising non-contact, non-invasive and low-cost examination to detect blood flow alterations, inflammation, and neurological dysfunction [3]. The aim of this study was to evaluate whether IRT can detect temperature changes in the distal forelimb (coronary band) of horses during partial vascular occlusion and re-perfusion induced by a pressure cuff. Fifteen adult healthy horses (12 geldings and 3 mares, mean age 9.3 years) underwent vascular occlusion of one forelimb using a cuff placed above the carpus. Horses were tested in two sessions under three conditions: (1) baseline (no cuff, 5 min duration), (2) occlusion (cuff inflated to 95% limb occluding pressure [4], 10 min duration), and (3) re-perfusion (cuff removed, 5 min duration). Images were acquired with a FLIR E76 thermal camera with an emissivity of 0.98, at 3 m in closed environment (stable). Three images per condition per session per horse were collected, resulting in a total of 9 images per session per horse and a total of 18 images per horse. Images were processed with FLIR Thermal Studio, defining an elliptical area on both coronets (Fig 1a). From each image, the mean temperature was measured from these areas and the delta temperature (occluded limb – not occluded limb) was calculated. A Repeated Measures General Linear Model (RGLM) analysed delta temperature with condition as the within-subject factor and session as the between-subject factor. Bonferroni post-hoc tests were applied.

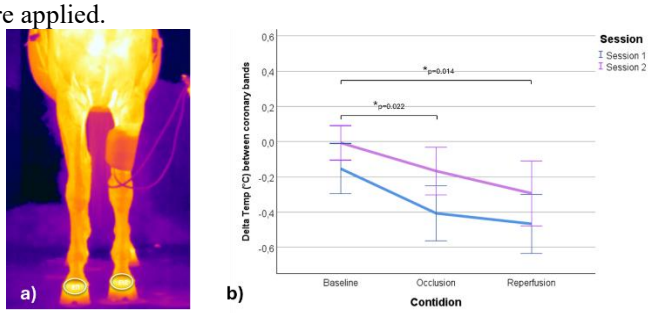


Figure 1 – a) Thermographic image showing the elliptical region of interest positioned over the coronary band for temperature calculation. b) Mean delta temperature across conditions in the two sessions. Error bars represent the 95th percentile. Significant differences are highlighted with asterisks.

Median delta temperature (°C) values decreased from baseline (Md=−0.10, 95th percentile=0.70) to occlusion (−0.35+/-0.80) and to re-perfusion (−0.35+/-0.70) (Fig 1b). Condition significantly affected delta temperature (RGLM, p=0.005), whereas session had no effect. Significant differences were found between baseline and occlusion (p=0.022) and between baseline and re-perfusion (p=0.014).These results indicate that IRT was useful in identifying a decrease in temperature due to a partial limb occlusion.

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Sequence-Informed Machine Learning to Predict Antigenic Distance Among Newcastle Disease Virus (NDV) Strains

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Newcastle disease virus (NDV) remains a major threat to poultry health and production despite widespread vaccination, largely because of rapid viral evolution and the recurrent emergence of antigenically divergent variants. Although sequencing is now fast and inexpensive, genetic distance alone is often a poor surrogate for antigenic relatedness and cross-protection, while haemagglutination-inhibition (HI) and neutralization studies are labor-intensive, slow, and constrained by logistics and strain availability. Here, we evaluated multiple machine learning (ML) strategies to predict NDV antigenic distance—measured by HI-based antigenic cartography—directly from viral sequence information.

A panel of vaccine and contemporary field strains representative of major circulating genotypes was propagated in embryonated SPF eggs, and hyperimmune sera were generated in SPF chickens. Cross-HI assays were performed and antigenic distances were quantified through antigenic cartography. Illumina sequencing was used to derive F (Fusion) and HN (Hemagglutinin-Neuraminidase) gene datasets. For each strain pair, we assembled predictors including nucleotide distance and site-specific amino-acid differences encoded with physicochemical descriptors (Atchley factors), after removal of invariant sites and highly correlated variables. Models were trained on 60% of the data with repeated 5-fold cross-validation (20 repeats) and evaluated on a fully independent 40% test set. We compared linear regression using genetic distance alone with bagging trees, random forest (RF), support vector machines, and artificial neural networks.

Across datasets, flexible ML models outperformed linear approaches. The best performance was achieved by an HN-based RF model, which provided substantially improved prediction of antigenic distance on the independent test set ($R^2 = 0.723$; RMSE = 0.509; MAE = 0.382) compared with the genetic-distance linear model ($R^2 = 0.051$; RMSE = 0.934; MAE = 0.753). Models based on F sequences were also predictive, consistent with shared evolutionary history between F and HN, while concatenating both genes did not consistently improve performance over HN alone. A subset of strains was additionally assessed by microneutralization to support the robustness of HI-derived antigenic patterns.

Overall, these results indicate that sequence-informed ML—particularly RF using site-level physicochemical features—can provide a rapid, objective proxy for NDV antigenic relationships, supporting timely vaccine strain selection and reducing reliance on resource-intensive experimental testing. The framework is readily transferable to other pathogens where genomic data and standardized antigenic or protection measures are available.

Subclinical ketosis as a driver of enteric greenhouse gas emissions in dairy cows

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Dairy cattle are a major contributor to greenhouse gas (GHG) emissions, primarily through enteric methane (CH_4) production [1]. While mitigation strategies have traditionally focused on diet formulation and genetics, the influence of metabolic disorders on enteric gas emissions has received limited attention [2,3]. Subclinical ketosis (SCK), a metabolic disease of early lactation, affects energy metabolism, feed efficiency, and rumen fermentation[4]. The aim of this study was to quantify the effects of SCK on enteric CH_4, carbon dioxide (CO_2), and hydrogen (H_2) emissions, as well as on production efficiency, in early-lactation dairy cows.

A total of 60 multiparous Holstein–Friesian cows were enrolled in a longitudinal study from 3 to 28 days in milk (DIM). Individual daily emissions of CH_4, CO_2, and H_2 were measured using an automated GreenFeed system. Blood β-hydroxybutyrate (BHB) concentrations were measured at 7, 14, 21, and 28 DIM to divide cows as healthy controls (CTR; BHB < 1.0 mmol/L at all time points; n = 43) or affected by SCK (KET; BHB ≥ 1.0 mmol/L at least once; n = 17). Statistical analysis was conducted with linear mixed models and $p \leq 0.05$ was used as significant.

Cows affected by SCK produced significantly more CH_4 than healthy cows, with average emissions of 402.8 ± 26.6 g/day in KET cows compared with 243.8 ± 17.7 g/day in CTR cows, corresponding to a 65% increase. When expressed as CO_2 equivalents (GWP_{100} = 27.2), CH_4 emissions amounted to 10.96 ± 0.72 kg CO_2e/day in KET cows *vs* 6.60 ± 0.48 kg CO_2e/day in CTR cows. The CH_4 intensity was markedly higher in KET cows, both per kilogram of milk (12.8 ± 1.2 *vs.* 7.7 ± 0.8 g CH_4/kg milk) and per kilogram of dry matter intake, indicating reduced metabolic and productive efficiency. In contrast, direct CO_2 emissions were lower in KET cows (11,035 ± 593 vs. 12,225 ± 416 g/day), and H_2 emissions were also reduced, suggesting enhanced H_2 utilization by methanogenic archaea. The analysis around the onset of SCK revealed a rapid increase in CH_4 emissions immediately after diagnosis, persisting for several days.

Despite lower CO_2 output, the higher CH_4-derived CO_2 equivalents resulted in a greater overall GHG footprint in cows affected by SCK. These results demonstrate that metabolic health is a key driver of enteric GHG emissions and highlight the potential of SCK prevention as a complementary strategy to improve both production efficiency and environmental sustainability in dairy systems.

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Comparison among different variables from a Laser Methane Detector (LMD) in a feeding trial with fattening bulls

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Methane (CH_4) is a greenhouse gas with a climate impact far greater than that of carbon dioxide [1]. In agriculture, CH_4 is produced mainly by enteric fermentation in livestock and during manure management [2]. Mitigation strategies such as feed additives can help lower CH_4 production. The aim of this study was to compare different measures of CH_4 concentration as attainable on a commercial beef cattle farm (all Charolaise breed males) using a portable Laser Methane Detector (LMD). Eighty-four of these bulls were selected for measurement using a randomised block design, with two parallel groups of 42 animals: a control group (C) and a treated group (T). The diet was administered *ad libitum* in the form of total mixed ration. The two groups differed with respect to inclusion in the supplement mix, in the T, of 10 g/head/d of a powder containing 10% of the blend of essential oils, bioflavonoids and tannins, and 90% of wheat bran. Animals in the treated group were given the supplement for 165 days. The animals were housed in twelve pens, with seven animals in each pen. According to Sorg et al. [3], the variables calculated on LMD raw data were arithmetic mean of all values (MEAN), arithmetic mean of all peaks (P_MEAN), arithmetic mean of all peaks belonging to eructation (ERP_MEAN), arithmetic mean of all peaks belonging to respiration (RES_MEAN), and the arithmetic mean of the maxima of each eructation event (EVENT_MEAN).

Spearman's correlations among the calculated items were higher between P_MEAN, ERP_MEAN, and EVENT_MEAN, whereas RES_MEAN had its higher correlation with MEAN. Within our results, RES_MEAN was the variable that better (significantly) changed because of dietary treatment. Our values (13.15 ± 1.20 vs 11.45 ± 1.17 ppm x m; $P < 0.05$) agree with those reported by Ricci et al. [4] and by Kang et al. [5]. In a Japanese study, the respiration data were correlated more significantly than the eructation ones with the CH_4 emissions determined with a gas analyzer [6]. Although in cattle more than 80% of the CH_4 is exhaled by eructation, the higher correlation coefficients obtained by that study suggest that respiration data may be more useful than eructation for quantifying the individual daily CH_4 emissions in cattle. In conclusion, further research reporting RES_MEAN will be useful to clarify the possible role of this item in the study of predictive equations for daily CH_4 emissions resulting from experimental dietary treatments.

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Measurement Systems and Policy-Oriented Software Tools for CH_4, CO_2 and NH_3 Emissions in Livestock Systems

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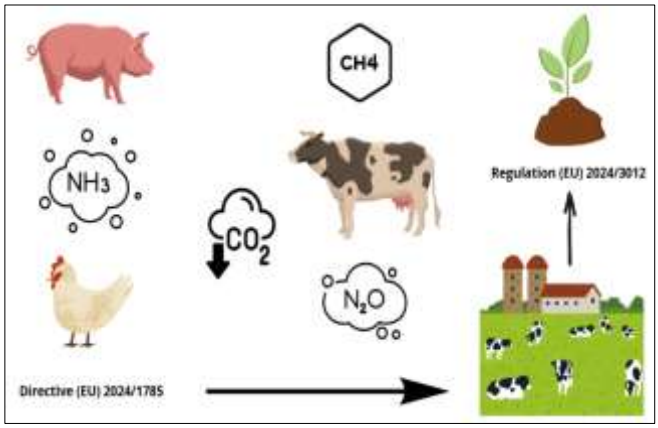


Figure - 1 - Livestock species and related greenhouse gas (CH_4, CO_2, N_2O) and ammonia (NH_3) emissions in the context of Directive (EU) 2024/1785 and Regulation (EU) 2024/3012.

The progressive strengthening of European environmental and climate legislation is redefining measurement and reporting requirements for greenhouse gases (CH_4, CO_2) and ammonia (NH_3) in livestock systems [1]. Livestock farming plays a strategic role in the sustainability transition; among production models, well-managed grazing-based systems have demonstrated the potential to enhance soil carbon sequestration, improve water-use efficiency, support biodiversity conservation, and contribute to climate change mitigation [2]. Recent regulatory developments, including Directive (EU) 2024/1785 [3] and Regulation (EU) 2024/3012 [4], reinforce the need for harmonised, transparent and technically robust emission quantification methodologies, particularly for intensive pig and poultry farms subject to Integrated Environmental Authorisation (AIA) thresholds defined in Livestock Units (LU). This study presents preliminary findings from a dual methodological approach combining: (i) an exploratory literature review conducted across major scientific databases (including Scopus and ScienceDirect), and (ii) a structured screening of European software tools supporting BAT (Best Available Techniques) compliance and emission accounting frameworks. The literature review confirms that most studies focus on pig and poultry systems, coherently with the regulatory scope of the Industrial Emissions Directive. Nutritional strategies such as a 2% reduction in crude protein (CP) have been shown to decrease daily nitrogen excretion without adversely affecting growth performance [4,5], contributing to more sustainable nitrogen cycle management [6]. At housing level, technological solutions including V-shaped scrapers and localised ventilation systems achieved reductions of 54% in NH_3 and 49% in N_2O emissions [7]. Regarding manure management, acidification and storage tank covering were associated with methane reductions up to 80% and ammonia reductions up to 70% [8]. Air scrubbers demonstrated removal efficiencies of up to 96% for volatile ammonia, while anaerobic digesters captured approximately 70% of methane generated from manure [9,6]. Despite these promising mitigation results, integration between direct gas measurement systems and IoT-based Precision Livestock Farming (PLF) architectures remains limited. Most studies rely on emission factors and inventory-based modelling approaches derived from the Intergovernmental Panel on Climate Change (IPCC) Guidelines for National Greenhouse Gas Inventories [10], instead of deploying interoperable, real-time sensor networks supported by uncertainty quantification and metrological traceability frameworks. The second analytical pillar examines European digital tools operationalising BAT principles for emission accounting. The Italian BAT-TOOL (developed under LIFE PREPAIR) [11] extends ammonia estimation for Directive (EU) 2024/1785 compliance to climate-altering gases following the IPCC 2006 Guidelines, covering housing, storage and land spreading phases. Comparable systems include Spain's Ecogan [12] and French emission labelling frameworks [13]. Considering these findings, the research examines the harmonisation potential of existing national digital emission-accounting tools and recent advances in PLF-based measurement systems, aiming to identify pathways towards greater methodological coherence across European livestock systems.

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Assessment of air quality and emissions in naturally ventilated pig houses through an IoT gas and environmental sensors device

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The uncontrolled presence of ammonia (NH_3) and carbon dioxide (CO_2) in pig houses is detrimental to animal health and the environment. Several factors influence air quality and emissions, including the frequency of slurry removal, floor type, weather conditions, and season [1]. This study monitored two naturally ventilated piggeries with different housing structures and slurry management systems: a fully slatted floor with a vacuum system for frequent slurry removal (Barn SFV) and a solid concrete floor in the indoor and outdoor areas of the pen (Barn CFO). Monitoring was conducted for two weeks in winter and summer using IoT sensor devices to continuously measure the concentration of NH_3, CO_2, temperature and humidity. Emissions were estimated via the indirect CO_2 mass balance method [2]. Gas levels and emissions were evaluated using mean hourly values over a 24-hour period. Regarding air quality, the average NH_3 and CO_2 concentrations in barn SFV were 14 ppm and 2850 ppm in winter and 6.3 ppm and 1060 ppm in summer, respectively; while in barn CFO they were 8.7 ppm and 1660 ppm in winter and 21.6 ppm and 1890 ppm in summer, respectively. The automated window management system of Barn SFV resulted in the accumulation of CO_2 and NH_3 during the winter, which was 2.7 times and 2.2 times higher than in the summer, respectively [3]. The barn CFO showed an increase in CO_2 in the winter 1.3 times higher than in the summer, but a reduction of NH_3 which was two times lower than in the summer. During the feeding distribution events, especially in the summer, NH_3 increased by up to 50% above the daily average value in the barn SFV, and by 76% in the barn CFO [3]. NH_3 emissions showed average values of 1.8 kg place^{-1} y^{-1} in winter and 3.2 kg place^{-1} y^{-1} in summer in the barn SFV, while in barn CFO the values were 2.3 kg place^{-1} y^{-1} in winter and 6.6 kg place^{-1} y^{-1} in summer. The highest emission levels were recorded during the hottest hours of the day in both seasons for both piggeries. The emission peak exceeded the daily average by 65% in the summer and 41% in the winter (barn SFV), and by 45% in the summer and 55% in the winter (barn CFO) [1,4]. Continuous air quality monitoring can help farmers better control the animal welfare, which aligns with the principles of precision livestock farming.

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Morphometric Variability of Cervical and Thoracic Circumferences in Holstein Dairy Cows Across Lactation Stage and Age

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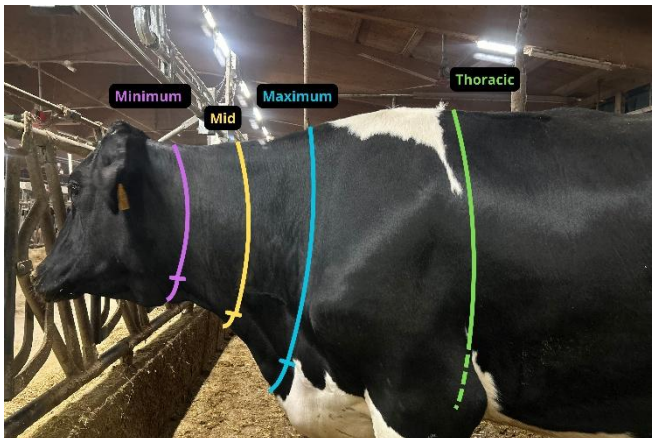


Figure 1. Schematic representation of cervical and thoracic circumference measurements in a Holstein dairy cow. Minimum neck girth (violet) was measured at the narrowest point near the head-neck junction, mid-neck girth (yellow) at the midpoint between minimum and maximum locations along the longitudinal axis of the neck, maximum neck girth (blue) at the most caudal portion of the neck, and thoracic girth (green) immediately caudal to the scapula. Measurements were taken with the cow standing in a natural, relaxed posture.

Neck morphometry in dairy cows remains poorly documented, despite its potential relevance for describing both physiological and structural variation across life stages **[1]** and its growing importance in the context of Precision Livestock Farming, where the neck represents a key anatomical site for sensor deployment **[2]**. This observational study aimed to characterise cervical and thoracic circumference variability in Holstein dairy cows and to examine their relationships with lactation stage (Days in Milk, DIM) and chronological age.

A total of 62 measurement events were collected from 35 Holstein cows housed under commercial-like conditions. Three cervical circumferences, minimum, mid-neck, and maximum, and thoracic girth were measured using a standardised protocol (Fig. 1). Body Condition Score (BCS), productive variables, and rumination data were recorded when available. Descriptive statistics were stratified by production group (heifers, lactating cows, dry cows), and non-linear polynomial models (degree 3) were used to explore associations with DIM and age.

Marked differences in cervical morphometry emerged among production groups. Minimum neck girth increased progressively from heifers (82.35 ± 5.61 cm [78.50–87.50]) to lactating cows (88.40 ± 4.94 cm [85.00–92.00]) and dry cows (94.00 ± 5.00 cm [91.00–97.00]). A similar pattern was observed for mid-neck girth, with mean values of 91.25 ± 4.30 cm [90.25–94.00] in heifers, 97.06 ± 5.68 cm [93.00–100.00] in lactating cows, and 101.33 ± 5.96 cm [98.25–104.00] in dry cows. Polynomial models showed significant non-linear associations with DIM for both minimum ($R^2 = 0.32$, RMSE = 4.26 cm, $P = 0.0066$) and mid-neck girth ($R^2 = 0.38$, RMSE = 4.68 cm, $P = 0.0017$).

Maximum neck girth displayed higher absolute values and greater variability (123.00 ± 12.88 cm in heifers; 145.18 ± 15.70 cm in lactating cows; 160.29 ± 15.32 cm in dry cows) and showed a weak association with DIM ($R^2 = 0.20$, $P = 0.0685$) but a stronger relationship with age ($R^2 = 0.41$, RMSE = 15.06 cm, $P < 0.0001$). Thoracic girth differed markedly between heifers (191.47 ± 7.95 cm [184.50–198.00]) and adult cows (>216 cm on average) and was strongly associated with age ($R^2 = 0.70$, RMSE = 8.76 cm, $P < 0.0001$), while showing only moderate variation across lactation.

The results demonstrate that cervical circumferences are region-specific traits influenced by distinct biological drivers. This study provides a quantitative, anatomically differentiated description of neck morphometry in dairy cows and establishes a preliminary framework for future, more detailed studies.

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Blood metabolomic characteristics in dairy cows treated with hepato-protectors during the transition period

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Metabolomics is an important branch of system biology that studies metabolites to elucidate disease etiology and characteristics, to monitor disease and develop biomarkers. The negative energy balance is characterized by body resources mobilization, altered oxidative state, inflammatory and immune responses. Amino acids (AA) shortage can compromise tricarboxylic acid (TCA) cycle leading to ketogenesis and hyperketonemia. Preventive protocol for hyperketonemia can supports liver metabolism (hepato-protectors) through AA, vitamins as cyanocobalamin, inositol (insulin-mimetic), and α-lipoic acid (antioxidant). The aim of this study was to identify blood metabolome in dairy cows treated with hepato-protectors preventive protocol.

A total of 60 Holstein-Friesian multiparous dairy cows from one farm were equally divided in (protocol number of ethical approvals 204359/2023): CTR or control group without treatment; and HEP or hepato-protectors group treated with two drugs, both administered at 12d pre-partum (pp) and 6d days in milk (DIM). The first one contained AA (acetyl-methionine, alanine, arginine, threonine, glutamate), inositol, and cyanocobalamin (IM 2 ml/10 kg BW); the second one contained acetyl-methionine, α-lipoic acid, and cyanocobalamin (IM 70 ml/animal). Both drugs were administered at 12d pre-partum (pp) and 6d days in milk (DIM). Blood sampling was performed from the coccygeal vein at -21 and -7d pp, and at 7, 14, 28 DIM for serum metabolomics analysis by 1H-NMR. Differences in metabolites were assessed with a linear mixed-effects model. A p-value<0.05 was accepted, whereas a 0.05≤p-value≤0.10 was considered as trend to significance.

The hyperketonemia prevalence was 10% in both groups at 7DIM, 23.3% in CTR and 16.7% in HEP at 14DIM, and 20% in CTR and 6.67% in HEP at 28DIM. A total of 50 metabolites were identified of which 26 differed by group (21 with p<0.05 and 5 with 0.05≤p≤0.10). The HEP had greater level of 13 metabolites compared to CTR (alanine, arginine, asparagine, choline, dimethyl-sulfone, glutamine, glycine, glyoxylate, histidine, methionine, myo-inositol, o-acetyl-carnitine, and taurine), and lower concentrations of 13 metabolites (2,3-butanediol, 3-hydroxybutyrate, 3-hydroxyisobutyrate, acetate, acetone, dimethylamine, isoleucine, leucine, methanol, methionine sulfoxide, succinate, TMAO, and valine). Metabolites' differences were 3.8% at 21pp, 42.3% at 7pp, 38.5% at 7DIM, 96.2% at 14DIM, and 80.8% at 28DIM. In conclusion, hepato-protectors improved the glucogenic AA, antioxidants, and fatty acid metabolism, with a probable improvement in the functioning of TCA cycle. Moreover, supplementation reduced the ketogenic AA and ketone bodies, markers of inflammation and oxidation, and ruminal methanogenic precursors, suggesting better management of the period.

Cyromazine contaminated substrate: effects on life-history characters, reproductive performances and heat resistance of *Hermetia illucens*

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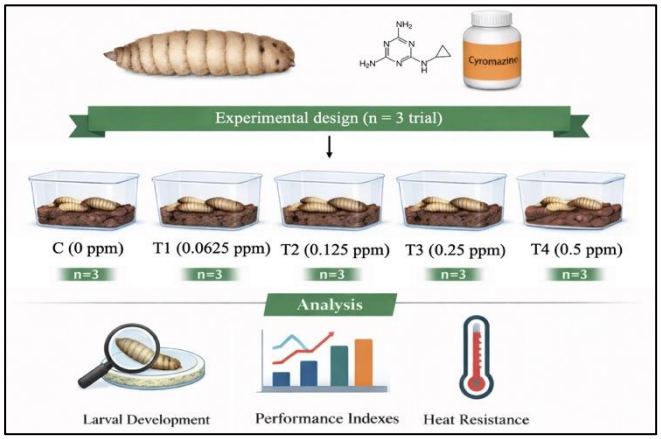


Figure 1 – Experimental design

Black soldier fly (BSF) (*Hermetia illucens*) has become a relevant species in the feed sector, due to its optimal nutritional profile and its ability to be reared on byproducts, thereby enhancing the sustainability of production systems [1, 2]. However, by-products are often poorly monitored [3], increasing the risk of substrate contamination, which may impair rearing performance and introduce contaminants into the food and feed chain [4]. In this study, we analysed the effects of cyromazine, an insect growth regulator, on BSF larvae. 1000 larvae (7 days old) were fed Gainesville diet (control, C) and four diets spiked with a cyromazine solution at different concentrations (0.0625 ppm, T1; 0.125 ppm, T2; 0.25 ppm, T3; 0.5 ppm, T4). The experiment was conducted three times, and each treatment was tested in triplicate each time (Figure 1). Larvae were reared until their weight variation remained within ±5% for three consecutive days. Growth performances were evaluated with the following indexes: feed conversion ratio frass corrected (FCR frass corrected), efficiency on conversion of ingested (ECI) and digested (ECD) feed. Moreover, at the end of each trial, a heat shock treatment was performed on 5 larvae per diet, to assess whether the contamination had detrimental effects on heat resistance. Larvae were individually placed in glass vials and immersed in a water bath with progressively increasing temperature, until complete cessation of movement (heat knockdown) was observed. Larval weight was significantly reduced only at the highest concentration of cyromazine, while no significant differences were observed between the control and the lower treatments (168.20±12.33, 177.52±9.88, 175.50±10.69, 161.72±12.96, and 110.15±38.70 mg respectively for C, T1, T2, T3 and T4; $P < 0.05$). Similarly, bioconversion indexes (FCR frass corrected, ECI, and ECD) were unaffected by the contaminant up to 0.25 ppm, with significant impairment occurring only at T4 (2.77±0.14, 2.89±0.33, 2.84±0.27, 3.22±0.74, 6.82±2.94 for FCR frass corrected, 0.36±0.02, 0.35±0.04, 0.35±0.04, 0.32±0.06, 0.16±0.02 for ECI and 0.38±0.02, 0.37±0.03, 0.38±0.04, 0.35±0.06, 0.21±0.06 for ECD, from C to T4; $P < 0.05$). As for the heat treatment, results have shown that heat resistance of larvae fed the two highest concentrations of cyromazine (T3 and T4) was significantly lower (49.67±0.95 and 49.16±0.75 °C) than C, T1 and T2 (50.13±0.52, 50.15±0.75 and 50.13±0.36 °C) ($P < 0.05$). According to these results, it is confirmed that cyromazine exerts negative effects on BSF larvae, therefore requiring a higher monitoring of substrates before administering, to ensure larval health and growth.

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Antiproliferative action of diketopiperazines extracted from vinery by-products in a sheep model

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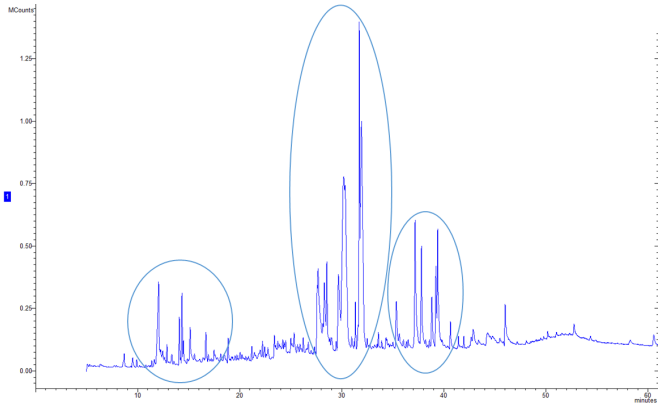


Figure 1 – Example of chromatogram of DKPs isomers (circle identifies the three main classes of isomers) obtained from wine lees treated with MAE method combined with water as an extraction solvent at 200°C.

By-products from grape processing are rich in bioactive compounds, such as phenols. Wine lees (WLs) are generated during the fermentation and ageing processes of wine [1]. Recently, a greener method for the extraction of bioactive compounds from WLs based microwave-assisted extraction (MAE) coupled with green solvents (ethanol and water) has been carried out [2]. In the present experiment, MAE coulpled with water as solvent have been combined to obtain a mixture of diketopiperazines (DKPs) isomers from WLs sampled from White (Wh), Rosè (Ro), and Red (Re) vinification, and their role on peripheral blood mononuclear cells (PBMCs) proliferation and viability have been tested during in vitro thermal stress. Extraction procedure was performed using MARS-6 microwave system, then the characterization was carried out with Gas chromatography-mass spectrometry using GC-MS/MS on a GC-MS/MS Ion Trap 240 system. PBMCs were isolated from dairy sheep peripheral blood using a gradient density method [3]. Cells ($2x10^6$) were activated with Concanavalian A (ConA) and Lipopolysaccharide (LPS) and six different DKPs enriched extracts containing two incremental content of DKPs (F1 and F2) were tested in an vitro thermal stress (37°C, 39°C to 43°C) at two concentration (0.4 and 0.8 mg/mL) on PBMC proliferative capacity using the incorporation of Bromodeoxyuridine during DNA sythesis. Moreover, a viability assay was performed. Data were analysed with the MIXED model of ANOVA using Turkey's post-hoc-test for multiple comparisons. Proliferation was significantly affected by in vitro thermal stress ($P<0.001$), by DKPs enriched extracts ($P<0.001$), and their interaction ($P<0.001$). In particular, all the DKPs enriched extracts reduced PBMCs proliferation in comparison with the positive control (PBMC stimulated with ConA and LPS). Furthermore, F2 extracts from all WLs vinery derived extracts (Wh, Ro, and Re) and tested at 0.4mg/mL showed an higher proliferative response than that at 0.8mg/mL. Interestingly, the F1 extracts from Wh, Ro, and Re WL tested at 0.4 and 0.8 mg/mL resulted in a proliferative response registered to the negative control (unstimulated PBMC). As regarding to the viability, DKPs enriched extracts improved the cell membrane integrity of cells; especially, F2 DKPs enriched extract from Re WL and tested at 0.8 mg/mL showed a higher cell membrane integrity than the positive control at both 39° and 43°C. Previous results demonstrated that DKPs extracted for the first time from WLs could modulate the proliferation of lymphomonocyes whitout affecting their viability, therefore, could represent interesting molecules for multiple application including both for human and animal purpose.

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Assessing liveweight dynamics and production responses to nutritional level in Sarda dairy ewes through walk-over-weighing technology

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In Mediterranean dairy sheep systems, seasonal feed shortages often result in periods of undernutrition, negatively affecting animal performance and welfare [1], particularly during lactation. Liveweight (LW) is a practical indicator of nutritional status and body reserve dynamics; however, conventional weighing is labour-intensive and usually performed at low frequency. Walk-over-weighing (WoW) systems integrated with electronic identification (EID) allow frequent, low-labour monitoring of individual animals and may improve the detection of nutritionally driven LW changes [2].

This study evaluated the sensitivity of a WoW system to detect moderate LW changes associated with contrasting nutritional levels in Sarda dairy ewes managed indoors or at pasture. Two experiments were conducted: (i) non-lactating ewes housed indoors and subjected to a short-term nutritional challenge; and (ii) lactating ewes managed on pasture with different daily access times and supplemented with hay and concentrate. In both experiments, ewes exposed to the lower nutritional level showed reduced LW, and the WoW system consistently detected these differences over time. Under grazing conditions during lactation, nutritional differences were also reflected in milk production indicators.

Overall, automated LW monitoring through WoW technology represents a feasible approach to track LW trajectories in dairy ewes under contrasting management conditions. Integrating LW data with routinely collected production traits (e.g. milk yield and composition) may support earlier identification of nutritional shortfalls and improve decision-making in welfare-oriented management.

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GPS-based assessment of pasture space use in Nera di Arbus sheep to support grazing management

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Effective grazing management requires the integration of multiple sources of information, including (i) animal nutritional requirements, (ii) herbage availability and quality, and (iii) the spatial extent of pasture accessible to grazing animals. In practical feeding plans, in the absence of spatial data, it is often implicitly assumed that grazing animals exploit the entire pasture area. However, this assumption is rarely verified empirically and may lead to an overestimation of the effective grazing surface used by the animals. We tested this assumption in a group of Nera di Arbus sheep, an autochthonous Sarda breed, using GPS tracking to quantify actual pasture space use and to provide operational indicators that can be integrated into feeding and grazing management. Spatial behaviour of grazing sheep was analysed at the Agris experimental farm (Macomer, Italy), within a 2.12 ha paddock stocked with 46 adult sheep (equivalent to 21.7 sheep ha^{-1}) during spring (19/03/2025 - 09/04/2025) and summer (26/06/2025 - 05/07/2025). Pasture use was quantified by estimating individual daily home ranges, through the 95% utilization distribution (UD95). For each animal and day, we calculated the area in which the individual was likely to be found with 95% probability using a dynamic Brownian bridge model [1], representing the portion of the pasture most regularly used. Seasonal differences in UD95 were assessed using mixed-effects models, with individual included as a random effect to account for repeated observations. Mean UD95 was 1.205 ± 0.069 ha (estimated marginal means ± SE) in March and 1.091 ± 0.127 ha in June, with no statistically significant difference between seasons ($p = 0.496$). When expressed relative to the total paddock size, sheep effectively used 56.8% of the available area in March and 51.5% in June. These results indicate that, even in a relatively small paddock, the effective grazing area used by sheep may be substantially lower than the total available surface. Overall, our findings highlight the importance of integrating GPS-derived spatial metrics with nutritional modelling and pasture assessment. Accounting for actual space use, rather than assuming full paddock utilization, may improve the realism of grazing management decisions and contribute to more accurate and sustainable feeding strategies in extensive and semi-extensive sheep systems.

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Effectiveness of dietary by-pass linseed oil on embryo-maternal relationship on day 14 of pregnancy in an ovine model

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PUFAS-ω3 have been studied in different tissues and systems [1,2]. Previous studies have demonstrated the positive effect of PUFAS-ω3 on ovarian function during the early stage of pregnancy in ewes, specifically on days 5-16 [3]. These studies have been performed using dietary by-pass linseed oil (LO; rich in PUFAS-ω3) in mated ewes. However, these trials did not demonstrate the effectiveness of LO on embryos from supplemented donor and their relationship with supplemented recipient ewes. Therefore, this study aimed to evaluate the effect of dietary LO on the embryo–maternal relationship until Day 14 of pregnancy (slaughter date).

Forty dry ewes (46.30 ± 3.90 months-old) were used in this study. Fourteen were used as embryo donor ewes, while twenty-six were used as recipient ewes. Seven donor and thirteen recipient ewes were fed using a control diet (CT; without LO) while the remaining donor and recipient ewes received a diet with microencapsulated LO (10.8 g of α-linolenic acid/ewe/day). Both CT and LO diets (concentrate and hay) were formulated to obtain similar energy and protein content. After an adaptation period, both diets were offered for 28 days [-14 to +14 days after mating day (Day 0)]. Body weight (BW) and body condition score (BCS) were measured and recorded before the onset of the trial and weekly until one day before the embryo transfer. Blood samples were taken on Days 5, 8, 11, and 14 (after mating) to determine P_4 levels in plasma. Two embryos from super ovulated donor ewes (supplemented/LOD and non-supplemented/ CTD) were transferred to recipient ewes (supplemented/LO and non-supplemented/CT) on day 7 after synchronized oestrous. Finally, the number of embryos recovered and corpora lutea were recorded on day 14 of pregnancy. Luteal function, in terms of progesterone (P_4) increasement, was assessed in recipient ewes. BW, BCS, and net energy intake were assessed using generalized lineal model. The number of embryos recovered and corpora lutea was analysed using Krukal-Wallis test. Robust linear regression (RLR) was performed for each group of recipient ewes with two embryos transferred to determine the relationship between P_4 levels and time (days). Statistical differences were established at $P<0.05$.

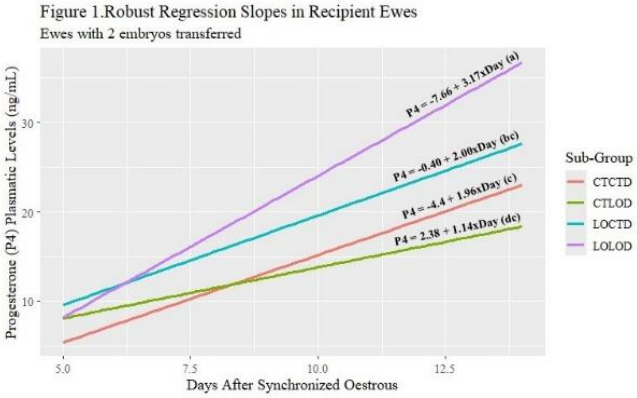


Figure 1 - Robust regression -slopes- of plasma progesterone levels between CTCTD: Recipient-CT ewes with transferred embryos from donor-CT ewes; CTLOD: Recipient-CT ewes with transferred embryos from donor-LO ewes; LOCTD: Recipient-LO ewes with transferred embryos from donor-CT ewes; LOLOD: Recipient-LO ewes with transferred embryos from donor-LO ewes. Different letters indicate statistically significant differences ($P<0.05$).

The means of BW, BCS, net energy intake/ewe, number of corpora lutea/ewe and embryo recovered/ewe on Day 14 were similar among groups of recipient ewes. However, RLR slopes showed that pregnant LO recipient ewes carrying embryos from LO donor ewes (LOLOD group) had a greater daily increase in P_4 concentrations than CT recipients carrying embryos from CT donors (CTCTD; $P=0.008$) or LO donors (CTLOD; $P<0.001$). Likewise, LOLOD ewes showed a higher daily increase in P_4 than LO recipients carrying embryos from CT donors ($P=0.011$; Figure 1).

In conclusion, LO supplementation positively affected maternal ovarian function. This effect could be enhanced by using embryos from supplemented donor ewes.

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