



2025 IEEE INTERNATIONAL WORKSHOP ON

Measurements and Applications in Veterinary and Animal Sciences

APRIL 28-30, 2025

PISA, ITALY



BOOK OF ABSTRACTS

WORKSHOP PROGRAM

Monday, April 28

Session A1.1 - Physiological Measurements of Animal Welfare - Part I

Room: Auditorium - Le Benedettine Conference Center

1 Non-Invasive Stress Monitoring in Domestic Dogs: a Multi-Parameter Approach Incorporating Body Temperature, Behaviour, and Hormonal Indicators

Giacomo Riggio (University of Pisa, Italy); Durmus Atilgan (University of Ankara, Turkey); Carmen Borrelli, Giulia Russo, and Chiara Mariti (University of Pisa, Italy)

2 Impacts of Animal Assisted Interventions on Elderly Wellbeing and Canine Welfare: a Multiparametric Assessment

Carmen Borrelli, Giulia Granai, Giulia Russo, Francesco Di Iacovo, Angelo Gazzano, Roberta Moruzzo, and Chiara Mariti (University of Pisa, Italy)

3 Smart Textiles to Assess Physiological and Emotional States in Horses During Positive Reinforcement Training

Martina Felici (University of Bologna, Italy); Angelo Gazzano, and Micaela Sgorbini (University of Pisa, Italy); Antonio Lanata (University of Florence, Italy); Aobh Gray and Adam R Reddon (Liverpool John Moores University, United Kingdom); Paolo Baragli (University of Pisa, Italy)

4 Body Condition Assessment in Sled Dogs: Intra- and Inter- Observer Agreement of BCS and Its Correlation with Ultrasonography and Plicometry

Sergio Maffi (Clinica Veterinaria Maffi, Italy); Alice Bonometti (Clinica Veterinaria La Quercia, Travagliato BS, Italy); Chiara Chiaffredo (Clinica Veterinaria Città di Torino, Italy); Andrea Galimberti (Clinica Veterinaria Caravaggio, Italy); Chiara Barletta (Clinica Veterinaria Fit Pet, Brembate BG, Italy); Laura Menchetti (University of Camerino, Italy); Alda Quattrone (University of Milano, Italy)

Session B1.1 - Machine Learning in Veterinary Science - Part I

Room: Room B - Le Benedettine Conference Center

5 Recognition of Horse Behaviours Using Deep Learning Techniques

Claudia Giannone (University of Bologna, Italy); Emanuela Dalla Costa, Chiara Maccario, Elie Atallah (Università Degli Studi di Milano, Italy); Marco Bovo (University of Bologna, Italy)

6 Exploring Saanen Lactation Curves Through Wood and Milkbot Models

Lucia Trapanese, Francesca Aragona, Giovanna Bifulco, Nadia Piscopo, Giuseppe Campanile, and Angela Salzano (University of Naples Federico II, Italy)

7 Use of Machine Learning Models for Detection of Dry off Intramammary Infections in Dairy Cows

Luca Barin, Giorgio Marchesini, and Paolo Balasso (University of Padua, Italy); Antonio Barberio, Lara Biasio, Erica Littamè, and Federico Martignago (Istituto Zooprofilattico Sperimentale delle Venezie, Italy); Henk Hogeveen and Mariska van der Voort (Wageningen University & Research, The Netherlands)

8 Assessing the Future Production Level of Dairy Cows with Machine Learning Tools

Marco Bovo (University of Bologna, Italy); Laura Ozella and Edoardo Fiorilla (University of Turin, Italy); Daniele Torreggiani and Patrizia Tassinari (University of Bologna, Italy)

9 Real-Time Classification of Ovine Pulmonary Diseases in Sheep Lungs Using AI-Powered CNN Models and Edge Computing

Muhammad Furqan Arshad (University of Sassari, Italy); Tesfalem Mehari Berhe (University of Pisa, Italy); Giovanni Lai (University of Sassari, Italy); Ilaria Fadda (IZS Sassari, Italy); Antonio Varcasia and Maria Luisa Pinna Parpaglia (University of Sassari, Italy); Riccardo Bazzardi (Istituto Zooprofilattico Sperimentale della Sardegna, Italy); Davide Adami (CNIT Pisa Research Unit, University of Pisa, Italy); Delaram Ghadir, Setayesh Ghadir and Stefano Giordano (University of Pisa, Italy); Ciriaco Ligios (Istituto Zooprofilattico Sperimentale della Sardegna, Italy); Giovanni Pietro Burrai (University of Sassari, Italy)

Session C1 - Measurement Techniques and Tools in Animal Reproduction

Room: Room C - Le Benedettine Conference Center

10 Assessment of Pelvic Diameter in Calves During Parturition Using Measurements of the Leg Distal Bone Diameters to Determine Delivery Method

Mirko Marracci, Duccio Panzani, Carmelo De Maria, Gianluca Caposciutti, Alice Buffi, and Bernardo Tellini (University of Pisa, Italy)

11 Performing Early Pregnancy Tests on Milk and Their Effect on Cow Welfare Compared to Rectal Pregnancy Tests 40 -45 Days Post-Insemination

Yaniv Lavon (Israel Cattle Breeders Association, Israel); Shmuel Friedman (Company & ISRAEL DAIRY BOARD, Israel)

12 Evaluation of a New Technological Device for Continuous Monitoring of Vital Functions in Newborn Foals

Maria Chiara Alterisio, Chiara Del Prete, Beatrice Mercaldo, Chiara Montano, Mariaelena De Chiara, Maria Pia Pasolini, and Paolo Ciaramella (University Federico II Naples, Italy)

Session A1.2 - Physiological Measurements of Animal Welfare - Part II

Room: Auditorium - Le Benedettine Conference Center

13 Hormonal Changes as Physiological Indicators of Welfare in Rabbit Does Supplemented with Omega-3

Alda Quattrone and Nour Elhouda Fehri (University of Milano, Italy); Stella Agradi (University of Torino, Italy); Laura Menchetti (University of Camerino, Italy); Olimpia Barbato (University of Perugia, Italy); Daniele Vigo (University of Milano, Italy); Luigi Turmalaj and Majlind Sulçe (University of Tirana, Albania); Marta Castrica (University of Padova, Italy); Maria Laura Marongiu (University of Sassari, Italy); Alessandro Dal Bosco (University of Perugia, Italy); Rafik Belabbas (Higher National Veterinary School, Algeria); Gerald Muça (University of Tirana, Albania); Fabio Gualazzi (University of Camerino, Italy); Gabriele Brecchia and Giulio Curone (University of Milano, Italy)

14 Metabolic Response and Growth Performance in Fattening Rab-Bits Supplemented with Linseed and Padina Pavonica Algae

Nour Elhouda Fehri (University of Milano, Italy); Stella Agradi (University of Torino, Italy); Laura Menchetti (University of Camerino, Italy); Olimpia Barbato (University of Perugia, Italy); Daniele Vigo and Giulio Curone (University of Milano, Italy); Marta Castrica (University of Padova, Italy); Simona Mattioli (University of Perugia, Italy); Sebastiana Failla and Michela Conto (CREA, Italy); Albana Munga (University of Tirana, Albania); Enkeleda Ozuni (University of Albania, Albania); Egon Andoni (University of Tirana, Albania); Fabio Gualazzi (University of Camerino, Italy); Bayrem Jemmali (University of Carthage, Tunisia); Sayed Abdel Kafi Mahfoz (Animal Production Research Institute, Egypt); Gabriele Brecchia and Alda Quattrone (University of Milano, Italy)

15 Hair Steroid Quantification: a Non-Invasive Method to Assess Reproductive and Welfare in Wild and Laboratory Species

Mattia Cesauri (Università di Pisa, Italy); Domenico Ventrella, Ilaria Troisio, Nadia Govoni, and Maria Laura Bacci (University of Bologna, Italy); Alberto Elmi (University of Pisa, Italy)

16 Animal Welfare Measures in the CAP 2023 - 2027: a Comparison of Member States with a Focus on Italian Outcomes During the First Year of Implementation

Giulia Pastorelli, Pietro Manzoni Di Chiosca Poggiolo, and Rita Iacono (CREA, Italy)

Session B1.2 - Machine Learning in Veterinary Science - Part II

Room: Room B - Le Benedettine Conference Center

18 An Innovative Approach to Raise and Manage Buffalo Herds

Valentina Russo (Logogramma, Italy); Lucia Trapanese (University of Naples Federico II, Italy); Martina Di Bratto and Marco Grazioso (Logogramma, Italy); Nicola Pasquino and Angela Salzano (University of Naples Federico II, Italy); Azzurra Mancini (Logogramma, Italy)

20 Leveraging Machine Learning and Explainable AI for Animal Health Assessment and Nutritional Analysis

Michele Magarelli (Università Degli Studi di Bari, Italy); Pierfrancesco Novielli and Donato Romano (Università Degli Studi di Bari Aldo Moro and INFN, Italy); Pierpaolo Di Bitonto (Università Degli Studi di Bari Aldo Moro, Italy); Sabina Tangaro (Università Degli Studi di Bari Aldo Moro and INFN, Italy)

21 Exploring Buffalo Lactation Curves with Clustering Algorithms

Nunzia Solmonte, Lucia Trapanese, Angela Salzano, and Nicola Pasquino (University of Naples Federico II, Italy)

Session C2.1 - Circularity and Implications on Sustainability Assessment in Breeding and Food Production: New Methods and Approaches - Part I

Room: Room C - Le Benedettine Conference Center

22 Assessing Water Scarcity in the Poultry Chain

Carlo Russo (University of Pisa, Italy); Giulio Mario Cappelletti and Filomena Chiara (University of Foggia, Italy); Pietro Russo (Data Analyst, Italy); Antonio Giovanni D'emilio (Department of Health Prevention, Italy); Giovanna Liguori (University of Naples, Italy)

23 Low-Cost IoT Sensors for Environmental Monitoring in Veterinary Applications

Alessandro Franco, Emanuele Crisostomi, and Carlo Bibbiani (University of Pisa, Italy)

24 Adopting Insect Meal in the Italian Trout Farming: a Multistage Process

Margherita Masi, Yari Vecchio, and Ernesto Simone Marrocco (University of Bologna, Italy); Marcello De Rosa (University of Cassino, Italy); Felice Adinolfi (University of Bologna, Italy)

26 Unlocking the Economic Potential of Neglected and Underutilized Fish Species as Food

Yari Vecchio and Margherita Masi (University of Bologna, Italy); Francesca Troise (University of Padova, Italy); Felice Panebianco and Tiziana Civera (University of Turin, Italy); Laura Prandini, Federica Savini, and Valentina Indio (University of Bologna, Italy); Valentina Terio, Elisabetta Bonerba, and Annamaria Pandiscia (University of Bari Aldo Moro, Italy); Leonardo Alberghini (University of Padova, Italy); Alessandra De Cesare and Andrea Serraino (University of Bologna, Italy); Federica Giacometti (University of Padova, Italy)

Session A1.3 - Physiological Measurements of Animal Welfare - Part III

Room: Auditorium - Le Benedettine Conference Center

28 Assessment of Plasma Concentrations of Procalcitonin (PCT), Protein Carbonylated Content (PCC), Symmetric Dimethylarg-Inine (SDMA) and Asymmetrical Dimethylarginine (ADMA) in Healthy Dairy Cows During Lactation

Giulia Sala, Giovanni Armenia, Chiara Orsetti, Matteo Castelli, Valentina Meucci, Lucia De Marchi, Micaela Sgorbini, and Francesca Bonelli (University of Pisa, Italy)

29 Impact of Milking Practices on the Welfare of Dairy Camels: a Behavioural and Physiological Approach

Marwa Brahmi (University of Sousse, Tunisia); Moufida Atigui (Institution de la Recherche et de l'Enseignement Supérieur Agricoles (IRESA), Tunisia); Mohamed Hammadi (Université de Gabès, Tunisia)

30 Optimization of Holstein Cow Lactation Curves in Tunisia: Im-Pact on Milk Production and Animal Welfare

Mohamed Amine Ferchichi and Safa Bejaoui (University of Carthage, Tunisia); Nour Elhouda Fehri (University of Milano, Italy); Laura Menchetti (University of Camerino, Italy); Gabriele Brecchia (University of Milan, Italy); Bayrem Jemmali (University of Carthage, Tunisia)

31 Monitoring Metabolic and Hormonal Changes in Sarda Rams Throughout the Breeding Season: a Key to Optimizing Their Management and Productivity

Francesca D. Sotgiu and Claudia Caporali (University of Sassari, Italy); Antonio Spezzigu (Embryosardegna, Tecnologia, Riproduzione e Fertilità, Perfugas, Sardinia, Italy); Matteo Sini, Andrea Mattu, Valeria Pasciu, and Francesca Mossa (University of Sassari, Italy); Pawel M Bartlewski (University of Guelph, Canada); Fiammetta Berlinguer (University of Sassari, Italy)

32 The Importance of Bovine Tuberculosis Genetic Resistance in Im-Proving Animal Welfare

Safa Bejaoui (University of Carthage, Tunisia); Nour Elhouda Fehri (University of Milano, Italy); Mohamed Amine Ferchichi (University of Carthage, Tunisia); Laura Menchetti (University of Camerino, Italy); Gabriele Brecchia (University of Milan, Italy); Bayrem Jemmali (University of Carthage, Tunisia)

33 Livestock Animal Hair as Indicator of Environmental Heavy Metals Pollution in Central Albania

Stella Agradi (University of Torino, Italy); Egon Andoni and Xhelil Koleci (University of Tirana, Albania); Enkeleda Ozuni (University of Albania, Albania); Rezart Postoli (University of Tirana, Albania); Laura Menchetti (University of Camerino, Italy); Gabriele Brecchia and Alda Quattrone (University of Milano, Italy); Mehmet Erman Or and Bengü Bilgiç (Istanbul University Cerrahpasa, Turkey); Duygu Tarhan (Bahcesehir University, Turkey); Ilir Dova (University of Tirana, Albania); Claudia Maria Balzaretto and Nour Elhouda Fehri (University of Milano, Italy); Ibrahim Ertugrul Yalcin (Bahcesehir University, Turkey); Albana Munga, Doriana Beqiraj and Pellumb Zalla (University of Tirana, Albania); Olimpia Barbato (University of Perugia); Giulio Curone (University of Milano, Italy); Marta Castrica (University of Padova, Italy)

Session B2 - Innovation, Precision and Creation: New Tools for Measurement and Visualization in Macroscopic and Microscopic Anatomy

Room: Room B - Le Benedettine Conference Center

34 How to Measure Neurons: a Pipeline to Characterize Their Shape and Morphology

Federico Tozzi and Ester Bruno (University of Pisa, Italy); Simone Cauzzo (University of Padua, Italy); Alejandro Luis Callara (Research Center "E. Piaggio", University of Pisa, Italy); Nicola Vanello, and Chiara Magliaro (University of Pisa, Italy)

35 Automatic Segmentation of Peripheral Nerve Histological Sections for Hybrid Models of Neuromodulation

Alice Giannotti and Claudio Verardo (Scuola Superiore Sant'Anna, Italy); Clement Albert (Ecole Polytechnique Federal de Lausanne, Switzerland); Giovanni Faoro (Scuola Superiore Sant'Anna, Italy); Chléa Schiff and Justine Bourgeot (Ecole Polytechnique Federal de Lausanne, Switzerland); Giulia Lazzarini, Andrea Pirone and Vincenzo Miragliotta (University of Pisa, Italy); Sara Moccia (Scuola Superiore Sant'Anna, Italy); Silvestro Micera (Scuola Superiore Sant'Anna, Italy & EPFL, Switzerland); Simone Romeni (EPFL, Switzerland)

36 Hybridizing Photogrammetry Techniques and AI to Produce a 3D Printing Realistic Replica of the Horse Colon

Anneliese Demil (University of Liege, Belgium); Etienne Levy (One Health Photography, France); Helene Leroy (University of Liege, Belgium)

37 Morphometric Analysis of Cerebellar Microglia in Male and Female Rats Using AI-Based Deep Learning

Giulia Lazzarini and Alessandra Gatta (University of Pisa, Italy); Alessia Cuccaro (Scuola Superiore Sant'Anna, Italy); Naif Onur Ceylan, Vincenzo Miragliotta, and Andrea Pirone (University of Pisa, Italy)

38 Deep Learning Based Approach to Quantify Cerebellar Neuronal Cell Loss in Feline Neurological Diseases: a New Potential Tool to Overcome Subjective Evaluation

Giulia Vadori (University of Padova, Italy); Valentina Vadori and Enrico Grisan (London South Bank University, United Kingdom); Maria Teresa Mandara (University of Perugia, Italy); Carlo Cantile (University of Pisa, Italy); Antonella Peruffo, Massimo Castagnaro, and Jean-Marie Graïc (University of Padova, Italy)

39 Deep Learning-Based Cell Segmentation on Nissl-Stained Histology Slices to Quantify Sexual Dimorphisms in the Cytoarchitecture of the Chimpanzee Cerebellum

Valentina Vadori (London South Bank University, United Kingdom); Jean-Marie Graïc (University of Padova, Italy); Antonella Peruffo, Livio Finos, and Giulia Vadori (University of Padova, Italy); Enrico Grisan (London South Bank University, United Kingdom)

40 Application of Surface Measurement Techniques in Veterinary and Animal Sciences: Integrating 3D Scanning, Photogrammetry, and 3D Printing

Luca Ceolotto, Jean-Marie Graïc, Tommaso Gerussi, and Sandro Mazzariol (University of Padua, Italy)

- 41 **Enhancing Histopathological Assessment: Automated Analysis of Bovine and Equine Optic Nerves**
Çağla Aytas, Giulia Lazzarini, Andrea Pirone, and Carlo Cantile (University of Pisa, Italy)

Session C2.2 - Circularity and Implications on Sustainability Assessment in Breeding and Food Production: New Methods and Approaches - Part II

Room: Room C - Le Benedettine Conference Center

- 42 **Sustainable Grass-Fed Supply Chain: the "Filerba" Case**
Giulio Mario Cappelletti (University of Foggia, Italy); Giorgio Mina (University of Turin, Italy); Carlo Russo (University of Pisa, Italy); Alessandro Bonadonna and Giovanni Peira (University of Turin, Italy)
- 44 **Soil Health and Circularity in Livestock Breeding**
Carlo Russo, Francesco Riccioli, and Carlo Bibbiani (University of Pisa, Italy); Giulio Mario Cappelletti (University of Foggia, Italy); Sergio Saia (University of Pisa, Italy)
- 46 **Towards Sustainable Mussel Farming: Innovative Practices and Perceptions of Italian Operators**
Ernesto Simone Marrocco, Gizem Yeter, Emanuele Dolfi, Margherita Masi, Yari Vecchio, and Felice Adinolfi (University of Bologna, Italy)
- 48 **ARMONIA: an Automated Remote MONitoring System of Nitrogen in Agriculture**
Alessandra Apostolico, Francesco Bonavolontà, Ester Scotto di Perta, Pierluigi Guerriero, Maria Teresa Verde, and Stefania Pindozi (University of Naples Federico II, Italy)
- 49 **Comparison Between Offshore and Inland Aquaculture from an Environmental Perspective**
Michele Zoli (University of Milan, Italy); Lorenzo Rossi, Carlo Bibbiani, and Baldassare Fronte (University of Pisa, Italy); Aurelie Wilfart, Christophe Jaeger, and Joël Aubin (INRAE, Italy); Alberto Pardossi (University of Pisa, Italy); Jacopo Bacenetti (University of Milano, Italy)
- 50 **Smart Monitoring and Control of the Environmental Conditions of a Swine Nursery Barn for Fossil-Free Heating**
Stefano Benni, Carlos Alejandro Perez Garcia, Claudia Giannone, Patrizia Tassinari, and Daniele Torreggiani (University of Bologna, Italy)

Tuesday, April 29

Session A2.1 - Innovative Approaches and Tools for Measuring Performances, Health, and Welfare in Farmed Animals - Part I

Room: Auditorium - Le Benedettine Conference Center

- 51 **Do the Largemouth Bass Like Live Insects? a Preliminary Study with Three Insect Species as Feed Enrichment**
Emma Copelotti (University of Pisa, Italy); Veronica Trabacchin and Sofia Violino (University of Padova, Italy); Simone Mancini (University of Pisa, Italy); Marco Birolo (University of Padova, Italy)
- 52 **Antimicrobial Resistance and Virulence Genes in Escherichia Coli Isolated from Young Healthy Chickens**
Giulia Cagnoli, Fabrizio Bertelloni, Margherita Marzoni Fecia Di Cossato, and Valentina Virginia Ebani (University of Pisa, Italy)
- 53 **Application of Digital Technologies to Promote Coexistence Between Wolves and Sheep Farms**
Alessia Di Rosso and Carlo Bibbiani (University of Pisa, Italy); Sandra Evans (Apidea Ltd, United Kingdom); Lucia Casini (University of Pisa, Italy); Huw Evans (Apidea Ltd, United Kingdom); Francesca Coppola, Chiara Benedetta Boni, and Antonio Felicioli (University of Pisa, Italy)

- 54 Potential Application of Precision Technology to Monitor Welfare and Interactions of Cage-Farmed Fish with Wild Fish Species**
Alessandra Roncarati, Martina Quagliardi, Gian Enrico Magi, Francesca Mariotti, and Livio Galosi (University of Camerino, Italy); Michele Di Iorio, Giusy Rusco, Emanuele Antenucci, Isabella Coccia, and Celeste Santoianni (University of Molise, Italy); Michela Cariglia (Consorzio Gargano Pesca, Italy); Luca Pozzato (Aquacloud, Polo Tecnologico Trentino Sviluppo, Italy); Nicolaia Iaffaldano (University of Molise, Italy)
- 55 Development and Validation of a Multi-Source Decision Support System for Cold Stress Management in Dairy Buffalo Farming, Including IoT Sensors and Milking Robot Data**
Maria Teresa Verde, Roberta Matera, Matteo Santinello, Francesco Bonavolontà, Lorenzo Coppola, Pierluigi Guerriero, and Gianluca Neglia (University of Naples Federico II, Italy)
- 56 Enhancing Dairy Goat Health Monitoring: Thermal Imaging for Heat Stress Detection and Behavioural Insights**
Martina Felici, Naod Thomas Masebo, Marilena Bolcato, Martina Zappaterra, Barbara Padalino, and Leonardo Nanni Costa (University of Bologna, Italy)
- 57 Evaluating Welfare, Milk Quality and Yield of Rendena Cows in Loose vs. Tied Housing Systems**
Silvia Sabbadin and Silvia Magro (University of Padova, Italy); Angela Costa (University of Bologna, Italy); Valentina Lorenzi, Francesca Fusi, and Luigi Bertocchi (Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Italy); Massimo De Marchi (University of Padua, Italy)
- 58 Dolfake: an Open-Source 3d Printed Dolphin Allowing the Standardization of Stranding Data Collection**
Etienne Levy (One Health Photography, France); Imane Taï (INRH, Morocco); Thierry Jauniaux (University of Liege, Belgium); Maylis Salivas (ACCOBAMS, Monaco)

Session B3.1 - Biologging in Veterinary and Animal Sciences: Measuring Physiology, Behaviour and Movement - Part I

Room: Room B - Le Benedettine Conference Center

- 59 From Wildlife to Domestic: How New Technologies Can Help Veterinary and Animal Sciences**
Carlo Catoni and Marco Cianchetti (Ornis italica & Technosmart Europe srl, Italy)
- 60 Exogenous Melatonin Alters Nocturnal and Diurnal Locomotor Activity, Body Temperature, Heart Rate, and Heart Rate Variability in Lambs, Measured by Triaxial Accelerometers and Subcutaneous Biologgers**
José-Alfonso Abecia (Universidad de Zaragoza, Spain); Carlos Palacios, Jaime Nieto and Javier Plaza (Universidad de Salamanca, Spain); Francisco Canto (Universidad de Zaragoza, Spain)
- 61 Smart Farming in Aquaculture: Using Biologgers to Improve Welfare and Yield**
Asgeir Bjarnason (Star-Oddi, Iceland)
- 62 Effect of Aquarium Environmental Enrichment in Welfare of Ornamental Fish (*Poecilia Reticulata*)**
Riccardo Crosetto, Elisabetta Macchi, Isabella Manenti, Martina Tarantola, Benedetto Sicuro, Patrizia Ponzio, Valentina Tafuro, and Cecilia Mugnai (University of Turin, Italy)
- 63 Cardiac Activity of Horses During Training Measured with External Polar Sensors**
Carlos Palacios, Santos Del Pozo, Jaime Nieto, and Javier Plaza (Universidad de Salamanca, Spain); José-Alfonso Abecia (Universidad de Zaragoza, Spain)
- 64 Unveiling Sleep in Horses: Monitoring Lying Behaviour in Different Housing Conditions**
Chiara Maccario (Università degli Studi di Milano, Italy); Elena Gobbo and Manja Zupan Šemrov (University of Ljubljana, Slovenia); Marco Bovo (University of Bologna, Italy); Elie Atallah, and Emanuela Dalla Costa (Università Degli Studi di Milano, Italy)
- 65 Monitoring Behavior in Heat-Stressed Dairy Buffaloes: General Pattern and Effect of a Nutritional Strategy**
Francesca Petrocchi Jasinski, Chiara Evangelista, and Loredana Basiricò (University of Tuscia, Italy); Miriam Iacurto, Roberto Steri, and David Meo Zilio (Council for Agricultural Research and Economics, Italy); Umberto Bernabucci (University of Tuscia, Italy)
- 66 Activity of Native Spanish Roosters (Castellana Negra and Pita Pinta) in Response to Hens of Different Breeds, Measured by Actigraphy**
Carlos Palacios, Jaime Nieto, and Javier Plaza (Universidad de Salamanca, Spain); José-Alfonso Abecia (Universidad de Zaragoza, Spain)

- 67 Precision Beekeeping Systems as Tools for Monitoring Bees to Agroecosystems Interactions**
Pier Paolo Danieli, Raffaello Spina, Riccardo Primi, Filippo Lazzari, Gloria Bernabucci, Federico Manganello, Paolo Viola, Pedro Girotti, and Bruno Ronchi (University of Tuscia, Italy)
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Session C3.1 - Metrology in Food Production Chain - Part I

Room: Room C - Le Benedettine Conference Center

- 68 Assessment of Plasma Treated Seawater (PTSW) in Shellfish Depuration Systems**
Annamaria Pandiscia, Eloisa Sardella, Patrizio Lorusso, Alessio Manfredi, and Valentina Terio (University of Bari Aldo Moro, Italy)
- 69 Valorisation of Neglected Fish Species Through the Dry-Curing Process: Microbiological Assessment of Thinlip Grey Mullet Fillets**
Francesca Troise (University of Padova, Italy); Felice Panebianco and Tiziana Civera (University of Turin, Italy); Laura Prandini, Federica Savini, Valentina Indio, Margherita Masi, and Yari Vecchio (University of Bologna, Italy); Valentina Terio, Elisabetta Bonerba, and Annamaria Pandiscia (University of Bari Aldo Moro, Italy); Leonardo Alberghini (University of Padova, Italy); Alessandra De Cesare and Andrea Serraino (University of Bologna, Italy); Federica Giacometti (University of Padova, Italy)
- 70 Analytical Measurement of Goat Cheese Aroma: Use of Electronic Nose Technology to Identify Products and to Value Their Quality**
Marica Egidio, Roberta Matera, Marika Di Paolo, Federica Pierro (University of Naples Federico II, Italy); Loriana Casalino (Universitas Mercatorum, Italy); Sophia Alesio and Raffaele Marrone (University of Naples Federico II, Italy)
- 71 Application of Next-Generation Sequencing Technologies to Insect-Based Products: Species Authentication and Microbiome Characterisation for Risk Assessment**
Gabriele Spatola, Alice Giusti, Simone Mancini, Lara Tinacci, Roberta Nuvoloni, Filippo Fratini, and Francesco Di Iacovo (University of Pisa); Laura Gasperetti (Istituto Zooprofilattico Sperimentale Lazio e Toscana, Italy); Andrea Armani (FishLab, Department of Veterinary Sciences, University of Pisa)
- 72 Using Cricket Flour (*Acheta Domesticus*) as an Additional Source of Protein in the Production of Freshwater Fish Patties**
Filomena Limone (University of Perugia, Italy); Sara Di Bella (IZSUM, Italy); Paolo Mantione (Alphafood s.r.l, Italy); David Ranucci, and Rossana Roila (University of Perugia, Italy); Maria Lucia Mercuri (IZSUM, Italy); Raffaella Franceschini (University Guglielmo Marconi, Italy); Raffaella Branciari (University of Perugia, Italy)
- 73 Determination of Iodine Content in Dairy Food Matrices**
Giovanni Niero, Marta Pozza, and Massimo De Marchi (University of Padova, Italy)
- 74 Measurement of Antioxidant Properties of Lactic Acid Bacteria (LABs) Isolated from Buffalo Milk**
Marika Di Paolo (University of Naples Federico II, Italy); Alejandra Cardelle Cobas (Universidad de Santiago de Compostela, Spain); Rosa Luisa Ambrosio, Marica Egidio and Alma Sardo (University of Naples Federico II, Italy); Carlos Manuel Franco Abuín (Universidad de Santiago de Compostela, Spain); Raffaele Marrone (University of Naples Federico II, Italy)
- 75 Controlling *E. Coli* STEC in Raw Milk Cheese: the Role of Curd Testing**
Giulia Cento, Giulia Weiss, Emanuela Cristelli, Katia Debiasi and Sonia Rodas (Istituto Zooprofilattico Sperimentale delle Venezie, Italy); Nicola Cologna, Alice Damaggio, Marco Nardelli, Pamela Piffer, and Marco Ramelli (Trentingrana Consorzio dei Caseifici Sociali Trentini, Italy); Rosaria Lucchini (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)
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Session A2.2 - Innovative Approaches and Tools for Measuring Performances, Health, and Welfare in Farmed Animals - Part II

Room: Auditorium - Le Benedettine Conference Center

- 76 A Smart Harness to Detect Attacks from Predators in Herds**
Luca Turini, Gianluca Caposciutti, Carlo Mancuso, Alice Buffi, Mirko Marracci, Bernardo Tellini, and Marcello Mele (University of Pisa, Italy)

77 Evaluating Welfare and Stress in Two Slow-Growing Local Poultry Breeds Versus a Commercial Hybrids in a Free-Range Farming System

Edoardo Fiorilla, Patrizia Ponzio, Elisabetta Macchi, Isabella Manenti, Barbara Miniscalco, Laura Ozella, and Cecilia Mugnai (University of Turin, Italy)

78 Evaluation of Milk Ejection Physiology in Italian Mediterranean Buffaloes Using Lactocorder

Roberta Matera and Federica Pierro (University of Naples Federico II, Italy); Lorenzo Pascarella (Associazione Italiana Allevatori, Italy & Università Cattolica del Sacro Cuore, Italy); Antonio Iraci Fuintino, Alessia Lombardi, and Giovanmaria Pacelli (University of Naples Federico II, Italy); Stefano Biffani (IBBA - CNR, Italy); Valentina Longobardi (University of Naples Federico II, Italy)

79 Electroencephalographic Analysis of Stunning Effectiveness in Donkeys at Slaughter

Beatrice Benedetti (University of Bologna, Italy); Katharine Ann Fletcher (Royal Veterinary College, United Kingdom); Mariano Hernandez Gil (National Autonomous University of Mexico, Mexico); Barbara Padalino (University of Bologna, Italy); Troy Gibson (Royal Veterinary College, United Kingdom)

Session B3.2 - Biologging in Veterinary and Animal Sciences: Measuring Physiology, Behaviour and Movement - Part II

Room: Room B - Le Benedettine Conference Center

80 Weaning Reduces Body Temperature and Heart Rate While Increasing Heart Rate Variability in Ewes

José-Alfonso Abecia (Universidad de Zaragoza, Spain); Asgeir Bjarnason (Star-Oddi, Iceland); Francisco Canto (Universidad de Zaragoza, Spain); Irene Viola, Isabella Manenti, Paola Toschi, and Silvia Miretti (University of Torino, Italy)

81 Measuring Activity and Space Use Overlap Between Domestic and Wild Ungulates in a Silvo-Pastoral System Using Camera Traps

Pedro Girotti, Paolo Viola, Gloria Bernabucci, Francesco Spada, Silvia Compagnucci, Carlo Maria Rossi, and Luciano Ortenzi (University of Tuscia, Italy); Luigi Esposito and Nadia Piscopo (University of Naples Federico II, Italy); Bruno Ronchi and Riccardo Primi (University of Tuscia, Italy)

82 An IoT-Driven Smart System for Rams' Physiological Status and Activity Monitoring

Delaram Ghadir, Setayesh Ghadir and Tesfalem Mehari Berhe (University of Pisa, Italy); Davide Adami (CNIT Pisa Research Unit, University of Pisa, Italy); Stefano Giordano (University of Pisa, Italy); Pietro Rossi (University of Veterinary Medicine, Sassari, Italy); Claudia Caporali, Antonio Spezzigu, Francesca D. Sotgiu, Francesca Mossa, and Fiammetta Berlinguer (University of Sassari, Italy)

Session C3.2 - Metrology in Food Production Chain - Part II

Room: Room C - Le Benedettine Conference Center

83 Measurements of Diet-Related Colour Changes in the Livery and Fillets of Gilthead Seabream: Focus on Quality Characteristics in the InsectFish Project

Emma Copelotti, Asia Zanzot, Chiara Sangiacomo, Roberta Moruzzo and Baldassare Fronte (University of Pisa, Italy); Giulia Andreani and Giovanni Sogari (University of Parma, Italy); Simone Mancini (University of Pisa, Italy)

84 Investigating the Microbiome of Acheta Domesticus and Locusta Migratoria-Based Food: a Preliminary Study

Anna Mottola and Chiara Intermite (University of Bari Aldo Moro, Italy); Roberta Piredda (Stazione Zoologica Anton Dohrn, Italy); Lucia Ranieri, Lucilia Lorusso, and Angela Di Pinto (University of Bari Aldo Moro, Italy)

85 Application of DNA Metabarcoding for the Botanical Traceability of Honey

Lucia Ranieri (University of Bari Aldo Moro, Italy); Roberta Piredda (Stazione Zoologica Anton Dohrn, Italy); Anna Mottola, Lucilia Lorusso, Chiara Intermite, and Angela Di Pinto (University of Bari Aldo Moro, Italy)

87 Boosting Industry Transparency and Sustainability Through Portable Long-Read Sequencing of Processed Seafood Products

Lucilia Lorusso (University of Bari Aldo Moro, Italy); Peter Shum (Liverpool John Moores University, United Kingdom); Anna Mottola (University of Bari Aldo Moro, Italy); Stefano Mariani (Liverpool John Moores University, United Kingdom); Roberta Piredda (Stazione Zoologica Anton Dohrn, Italy); Chiara Intermite, Lucia Ranieri and Angela Di Pinto (University of Bari Aldo Moro, Italy)

Session A2.3 - Innovative Approaches and Tools for Measuring Performances, Health, and Welfare in Farmed Animals - Part III

Room: Auditorium - Le Benedettine Conference Center

89 Bio-Acidification for Mitigating Ammonia Emissions in Dairy Barns: a Preliminary Laboratory Study

Serena Vitaliano, Grazia Cinardi, Provvidenza Rita D'Urso, Luciano Manuel Santoro, Emanuele La Bella, Rossella Saccone, Ferdinando Fragalà, Alice Finocchiaro, and Giovanni Cascone (University of Catania, Italy)

90 Zebrafish from Human to Veterinary and Animal Sciences: a Key Model for Measuring Physiology, Behaviour, and Nutritional Interventions

Rosario Licitra, Valentina Gazzano, Carlo Bibbiani, Chiara Sangiacomo (University of Pisa, Italy); Mahanama De Zoysa (Chungnam National University, Korea); Zivile Jurgelene (State Scientific Research Institute Nature Research Centre, Lithuania); Filippo Santorelli and Maria Marchese (IRCCS Stella Maris Foundation, Italy); Alessandro Cellerino and Letizia Brogi (Scuola Normale Superiore, Italy); Baldassare Fronte (University of Pisa, Italy)

92 APPàre: the Digital Platform for Innovation in the Sardinian Livestock Sector

Matteo Sini, Andrea Mattu, Pietro Masala, and Antonio Spezzigu (University of Sassari, Italy); Nicoletta Deiana and Tiziana Fanni (Abinsula srl, Italy); Riccardo Bazzardi and Ciriaco Ligios (Istituto Zooprofilattico Sperimentale della Sardegna, Italy); Fiammetta Berlinguer (University of Sassari, Italy); Katiuscia Zedda (Abinsula srl, Italy)

93 Impact of Cold Stress on Daily Milk Production in Italian Mediterranean Buffaloes

Matteo Santinello, Maria Teresa Verde, Federica Pierro, Francesco Bonavolontà, Lorenzo Coppola, Pierluigi Guerriero, and Gianluca Neglia (University of Naples Federico II, Italy)

Session B4 - Innovative Technologies for Parasite and Vector Control

Room: Room B - Le Benedettine Conference Center

94 Evaluation of Coccimorph Image-Based Software as a Complementary Tool for the Identification of Chicken Eimeria Species in Field Samples

Marta Raffaelli and Valentina Meucci (University of Pisa, Italy); Alessia Zoroaster (University of Padova, Italy); José Jaramillo-Ortiz (Royal Veterinary College, United Kingdom); Antonio Frangipane Di Regalbono (University of Padova, Italy); Damer Blake (Royal Veterinary College, United Kingdom); Stefania Perrucci (University of Pisa, Italy)

95 Optimizing Current Approach to Control Cystic Echinococcosis Using Drone Technology and Geospatial Tools

Nicola Lattero, Martina Nocerino, Paola Pepe, Antonio Bosco, Elena Ciccone, Maria Paola Maurelli, and Laura Rinaldi (University of Naples Federico II, Italy)

96 Open-Source Toolkit for in Vitro Diagnosis of Malaria Tailored for Low-Resource Settings

Florinda Coro, Carmelo De Maria, Valentina Mangano, and Arti Ahluwalia (University of Pisa, Italy)

97 Integrating Engineering and Public Health to Monitor Waterborne Intestinal Pathogens: a One Health Approach

Vincenzo Mignano, Antonino Pace, Paola Pepe, Federica Ceraso, Ludovico Di Pineto, Laura Rinaldi, and Massimiliano Fabbicino (University of Naples Federico II, Italy)

98 Innovative Technologies for Diagnosis of Intestinal Parasites in Animals and Humans

Maria Paola Maurelli and Salvatore Capuozzo (University of Naples Federico II, Italy); Giuseppe Martone (Universitas Mercatorum, Italy); Paola Vitiello, Paola Pepe, Antonio Bosco, Mirella Santaniello, Michela Gravina and Stefano Marrone (University of Naples Federico II, Italy); Biase Celano (Officina Elettronica SPA, Italy); Carlo Sansone, Giuseppe Cringoli, and Laura Rinaldi (University of Naples, Federico II, Italy)

99 Risk Assessment of (Re)-Emerging Vector-Borne Diseases in Southern Europe Based on Machine Learning Processing of Poly-Observational Data

Monica Falcinelli, Claudia Damiani, Alessia Cappelli, Irene Ricci, and Guido Favia (University of Camerino, Italy)

Session C4 - Precision Nutrition Technologies in Animal Husbandry: Advancements and Future Perspectives

Room: Room C - Le Benedettine Conference Center

100 The Use of Ruminant Boluses to Detect Bouts in Beef Cattle

Davide Martini and Silvia Magro (University of Padova, Italy); Angela Costa and Martina Lamanna (University of Bologna, Italy); Clotilde Villot, Eric Chevaux and Bruno Martin (Lallemand Animal Nutrition, France); Alberto Guerra (University of Padova, Italy); Matteo Santinello (University of Naples Federico II, Italy); Massimo De Marchi (University of Padua, Italy)

102 Heart Girth as a Predictor of Body Weight in Lactating Cows

Silvia Magro, Alberto Guerra, Mauro Penasa, and Massimo De Marchi (University of Padova, Italy)

103 Using Solomon Coder for the Ethological and Feeding Behaviour Analysis of Dairy Cows in Tie-Stall Housing: a Practical Guide

Martina Lamanna, Claudia Giannone, Marco Bovo, and Giacomo Bellisola (University of Bologna, Italy); Alberto Romanzin (University of Udine, Italy); Damiano Cavallini (University of Bologna, Italy)

104 Eating Time of Growing Simmental Bulls Evaluated with Different Approaches

Matteo Braidot and Anita Cabbia (University of Udine, Italy); Damiano Cavallini (University of Bologna, Italy); Mauro Spanghero and Alberto Romanzin (University of Udine, Italy)

105 Impact of Different Feeding Strategies on Carcass Characteristics, Meat Quality, and Organoleptic Properties of Meat from Old Sheep for Arrostiticini Production

Daria Zoe De Angelis, Luigi Pompei, Hassan Jalal and Paraskevya Prasinou (University of Teramo, Italy); Mirella Bellocchi, and Leopoldo Di Luca (Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy); Isa Fusaro (University of Teramo, Italy)

Session A3 - Mass spectrometry as a cutting-edge tool in veterinary and animal science

Room: Auditorium - Le Benedettine Conference Center

106 Liquid Chromatography-High Resolution Mass Spectrometry Analysis of Milk as a Non-Invasive Matrix for Assessing Dairy Cows' Exposure to Perfluoro-Alkyl Substances in Italy

Federica Di Cesare and Susanna Draghi (University of Milan, Italy); Pavlovic Radmila (IRCCS San Raffaele Scientific Institute, Italy); Giulio Curone, Petra Cagnardi (University of Milan, Italy); Alberto Pellegrini and Marco Fidani (UNIRELAB Srl, Italy); Francesco Arioli (University of Milan, Italy)

107 Advancing Veterinary Medicine with LC-MS/MS: Applications in Pharmacology, Toxicology, and Precision Diagnostics

Anisa Bardhi and Andrea Barbarossa (University of Bologna, Italy)

108 Salinity-Driven Alterations in Tetracycline Exposed *Mytilus Galloprovincialis*: a Metabolomics Perspective

Alessio Lenzi (University of Pisa, Italy); Marta Cunha, Constança Figueiredo, and Carla Leite (University of Aveiro, Portugal); Lucia De Marchi, Gianfranca Monni, and Valentina Meucci (University of Pisa, Italy); Tania Russo and Gianluca Polese (University of Naples Federico II, Italy); Amadeu Soares and Rosa Freitas (University of Aveiro, Portugal)

109 1H NMR for Comparative Metabolic Analysis of Whey and WPC-80

Ingrid Maria Sousa Gurgel do Amaral (University of Padova, Italy); Gaia Meoni and Leonardo Tenori (University of Florence, Italy); Marta Pozza, Massimo De Marchi, and Giovanni Niero (University of Padova, Italy)

Session C5 - Measuring Microplastics (MPs) and Nanoplastics (NPs) in the Livestock Sector

Room: Room C - Le Benedettine Conference Center

110 Development of Portable NIR Calibration Models for Micro-Plastic Detection and Quantification in Corn Silage

Federico Manganello and Pier Paolo Danieli (University of Tuscia, Italy); Abid Khalil and Salvatore Barbera (University of Turin, Italy); Rabeb Issaoui (University of Tunis El Manar, Tunisia); Hatsumi Kaihara and Sonia Tassone (University of Turin, Italy)

111 Microplastics Measurements in Cow Milk: Extraction and Characterization Methodologies

Valentina Balestra, Monica Granetto, and Rossana Bellopede (Politecnico di Torino, Italy); Sonia Tassone (University of Turin, Italy)

112 Enhancing Microplastic Detection Directly in Feeds Using Ghost NIR Spectra

Salvatore Barbera (University of Turin, Italy); Giorgio Masoero (Accademia di Agricoltura di Torino, Italy)

Wednesday, April 30

Session A4 - Advanced Technologies and Measurement Tools for Clinical Diagnostic Procedures in Veterinary Medicine

Room: Auditorium - Le Benedettine Conference Center

113 Evaluation of Learning Curve for Point-of-Care Ultrasound Protocol in Equine Species: Preliminary Study

Dania Cingottini (University of Pisa, Italy); Irene Nocera (Scuola Suuperiore Sant'Anna, Italy); Benedetta Sorvillo and Giulia Sala (University of Pisa, Italy); Soren Boysen (University of Calgary, Canada); Francesca Bindi, Francesca Bonelli, and Micaela Sgorbini (University of Pisa, Italy)

114 Reliability of a Digital Stethoscope Associated with Phonocardiography for Cardiac Auscultation in Dogs

Lisa Alibrandi (Sant'Anna School of Advanced Studies, Italy & Veterinary Science, University of Pisa, Italy); Nicole Parrucci, Giovanni Grosso, Rosalba Tognetti, and Tommaso Vezzosi (University of Pisa, Italy)

115 Applicability of Two-Dimensional Shear Wave Elastography for Assessing Hepatic Stiffness in Dogs with Right-Sided Congestive Heart Failure

Caterina Puccinelli, Pelligra Tina, Tommaso Vezzosi, and Simonetta Citi (University of Pisa, Italy)

116 Smart Textile Biotechnology for ECG Monitoring in Minipigs: a Validation Study

Aurora Paganelli (Scuola Superiore Sant'Anna di Pisa, Italy); Emma Tognetti (University of Pisa, Italy); Martina Felici (University of Bologna, Italy); Antonio Lanata (University of Florence, Italy); Fabio Anastasio Recchia (National Research Council, Italy); Angelo Gazzano and Micaela Sgorbini (University of Pisa, Italy)

117 Electrocardiographic Recording and Sharing Using Digital Devices in Equids

Chiara Bozzola, Lorenzo Schinardi, and Enrica Zucca (University of Milano, Italy)

118 Pilot Study for the Feasibility of Deep Photoacoustic Imaging in Pig Bladder Throughout Developed Laser Catheter Consumable

Chiara Di Franco (CNR-Institute of Clinical Physiology, National Research Council, Pisa & University of Pisa, Italy); Paolo Armanetti (Institute of Clinical Physiology, Italian National Research Council, Italy); Cosimo Trono (Istituto di Fisica Applicata Nella Carrara CNR-IFAC, Italy); Jose Jithin (FUJIF ILM Visua ISONICS, Amsterdam, Belgium); Mauro Comes Franchini (University of Bologna, Italy); Massimo Alfano (Division of Experimental Oncology Unit of Urology Ospedale San Raffaele, Italy); Luca Menichetti (National Council of Research, Institute of Clinical Physiology, Pisa, Italy)

119 Computed Tomography (CT) Scan Measurements for the Assessment of the Appropriate Tidal Volume to Apply in Mechanically Ventilated Rabbits Under General Anesthesia

Luca Pennasilico (University of Camerino, Italy); Elisa Silvia D'urso (Mindray company, Italy); Paolo Selleri (Centro Veterinario Specialistico, Italy); Caterina Di Bella (University of Camerino, Italy)

120 Validation of a Novel Technological Device for Vital Functions Monitoring in Hospitalized Dogs

Beatrice Mercaldo, Maria Chiara Alterisio, Antonio Di Loria, Jacopo Guccione, Camilla Sangiuliano, Fabiana Micieli, Sergio Esposito, and Paolo Ciaramella (University of Naples Federico II, Italy)

Session B5 - The importance of Measurements and Indices in Cyto and Histopathology of Tissues from Companion and Farmed Animals

Room: Room B - Le Benedettine Conference Center

121 Assessing the Health of Farmed Rainbow Trout (*Oncorhynchus Mykiss*): Use of an Open-Source Software for Intestine Histology

Martina Quagliardi, Livio Galosi, Francesca Mariotti, Alessandra Roncarati, and Gian Enrico Magi (University of Camerino, Italy)

122 Measurement of Intestinal Morphology, Growth Rate, and Immunity Response in Nile Tilapia (*Oreochromis Niloticus*) Fed with Slab51 Probiotic Mixture

Danilo De Bellis, Lucia Biagini, and Sara Berardi (University of Camerino, Italy); Mateo Espejo Valencia and Gilberto Gonzalez Aguirre (Universidad Tecnologica de Pereira, Colombia); Giacomo Rossi (University of Camerino, Italy)

123 Reference Intervals and Innovative Parameters Using Sysmex XN-2000V in Horses

Chiara Masci (University of Camerino, Italy); Caterina Fani (CDVet, Italy); Martina Quagliardi, Giacomo Rossi, and Alessandra Gavazza (University of Camerino, Italy)

124 Morphometric and Histopathological Evaluation of Chickens' Immune Organs Following in-Ovo Probiotic and Postbiotic Administration

Lucia Biagini, Livio Galosi, Danilo De Bellis, Valentina Grifantini, Subeide Mari, and Giacomo Rossi (University of Camerino, Italy)

125 A New Approach to Evaluate Veterinary Histopathological Tissue Sample with Image and Ultrasound Data

Giacomo Rossi (University of Camerino, Italy); Marton Csaba Mezei, Ilaria Benedetti and Fabrizia Auletta, and Calogero Maria Oddo (Scuola Superiore Sant'Anna, Italy); Alessandra Gavazza (University of Camerino, Italy)

126 Digital Immunohistochemical Quantification of Neu5Gc Expression in Canine Intestinal Biopsies and Its Correlation with Colitis Severity

Maria Chiara Muollo, Lucia Biagini, Daniele Tomassoni, Vincenzo Bellitto, Danilo De Bellis, and Giacomo Rossi (University of Camerino, Italy)

Session C6 - Application of Measurement Concepts in Veterinary Microbiology and Infectious Diseases

Room: Room C - Le Benedettine Conference Center

127 Prevalence and Antimicrobial Resistance Profiles of Bacteria Recovered from Wounds of Stray Dogs and Cats Before and After Washing with Sterile Physiological Solution

Francesca Paola Nocera, Sinem Arslan, Rossana Schena, and Francesca Pizzano (University of Naples Federico II, Italy); Cristina Di Palma (Azienda Sanitaria Locale Napoli 3 Sud, Italy); Silvia Cappiello and Marina Pompameo (Veterinary Hospital, ASL Napoli 1 Centro, Italy); Barbara Lamagna, and Luisa De Martino (University of Naples Federico II, Italy)

128 Methodological Framework to Study the Antibiotic Resistance Evolvability of Pathogenic Staphylococcal Species

Elisa Rampacci, Tommaso Felicetti, Francesco Biretoni, Rolando Arcelli, Riccardo Zelli, Stefano Sabatini, and Fabrizio Passamonti (University of Perugia, Italy)

- 129 Challenges in Measuring the Antimicrobial Activity of Natural Compounds in in Vitro Tests**
Valentina Stefanetti, Elisa Rampacci, Patrizia Casagrande Proietti, David Ranucci, and Fabrizio Passamonti (University of Perugia, Italy)
- 130 Proposal of a Risk-Based Approach for Defining the Calibration Frequency of Remote Temperature Sensors in Use at the Laboratories of Istituto Zooprofilattico Sperimentale Delle Venezie (IZSve)**
Silvia Benato, Laura Trevisan, Riccardo Muliari, Paola Carnieletto, Lavinia Guerra, and Laura Zanovello (Istituto Zooprofilattico Sperimentale delle Venezie, Italy)
- 131 Development of an Autogenous Dip Vaccine Against Furunculosis (*Aeromonas Salmonicida*) as Tool for Animal Precision Medicine and Management: Preliminary Data**
Giulio Severi, Martina Pellegrini, Giulia Vita, Chiara Ovidi, Silvia Cardaioli, Massimo Bugatti, and Monica Cagiola (IZSUM, Italy); *Ciro Castellano (Eredi Silvio Rossi, Italy); Antonella Di Paolo (Istituto Zooprofilattico sperimentale dell'Umbria e delle Marche Togo Rosati, Italy); Laura Boriani, Eleonora Radicchi, Rosario Liberti, Lucia Anzalone, Martina Torricelli, and Francesco Agnetti (IZSUM, Italy)*

Session C7 - Radio Frequency Technologies for Animal Identification and Tracking

Room: Room C - Le Benedettine Conference Center

- 133 RFID-Enabled Smart Livestock Management Systems**
Debi Dutta, Marcello Mele, and Filippo Costa (University of Pisa, Italy)
- 134 Battery-Less Implantable Wireless Sensor for Proactive Fever Monitoring in Livestock**
Nicoletta Panunzio (University of Rome Tor Vergata, Italy); Carolina Miozzi (University of Rome "Tor Vergata", Italy & Radio6ense Srl, Italy); Gaetano Marrocco (University of Rome Tor Vergata, Italy)
- 135 UHF-RFID Localization for Sheep Monitoring in Milking Parlour**
Alice Buffi, Luca Turini, Gianluca Caposciutti, Mirko Marracci, Bernardo Tellini, and Marcello Mele (University of Pisa, Italy)

Non-Invasive Stress Monitoring in domestic dogs: A Multi-Parameter Approach Incorporating Body Temperature, Behaviour, and Hormonal Indicators

Giacomo Riggio¹, Durmus Atilgan², Carmen Borrelli¹, Giulia Russo*¹, Chiara Mariti¹

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Both behavioural and physiological parameters are commonly utilized in welfare assessment, although their combination sometimes shows discrepancies. This study aimed to understand if a correlation exists between behavioural stress signs and physiological parameters commonly used in dog welfare assessment.

Sixty-seven caregiver-dog dyads were involved. All the dogs (41 females and 26 males) were healthy, with a mean age of 6.0 ± 3.2 years (min=1; max=14) and belonging to various breeds.

All dyads underwent the Strange Situation Procedure (SSP), a mildly stressing procedure, in which the occurrence of 11 stress behaviours (shaking, nose/lip licking, paw lifting, self-scratching/grooming, yawning, whining/yelping, barking, stretching, escape attempts, locomotion and door sniffing) and the total duration of locomotion were recorded. Seven minutes after SSP (T1), a mock veterinary visit was carried out, in which salivary samples, heart rate, blood pressure and body temperature were collected from each dog. Salivary samples collected before (T0) and after the test (T1) were used to measure delta cortisol levels. A Spearman's Correlation test ($p < 0.05$) was used to assess possible relationships between behavioural, physiological and hormonal parameters; and a Mann-Whitney U test ($p < 0.05$) to compare body temperature between different seasons.

No significant difference ($p > 0.05$) in body temperature was found between dogs tested with higher (April-September; mean 39.0°C) and lower (October-March; mean 38.7°C) environmental temperatures, so data on body temperature were merged in one group. Delta salivary cortisol concentrations (T1-T0) were calculated, the median value resulting in 0.024 ng/mL , while the median occurrence of stress signals was 72 (min=21; max=287).

According to the Spearman's correlation test body temperature showed a fairly positive association with both stress signals ($\text{Rho} = 0.29$, $p < 0.05$) and delta cortisol concentrations ($\text{Rho} = 0.28$, $p < 0.05$).

Based on the results obtained, the higher occurrence of stress signals and higher cortisol delta values are associated with increased body temperature. Body temperature, whether as one of multiple physiological indicators or as the only measure obtained with infrared cameras, is sometimes utilized in stress assessment when a non-invasive approach is required, such as in zoo or wildlife contexts. Our results support the use of body temperature as one of the key stress indicators, especially combined with other indicators, and suggest the potential of infrared thermography as a non-invasive tool for real-time evaluation of the subject's welfare status.

Given the growing interest in this technology for pets and the many variables influencing its effects beyond stress, further research remains crucial.

- Riggio, G.; Borrelli, C.; Campera, M.; Gazzano, A.; Mariti, C. Physiological Indicators of Acute and Chronic Stress in Securely and Insecurely Attached Dogs Undergoing a Strange Situation Procedure (SSP): Preliminary Results. *Vet.Sci.* 2022, 9, 519. <https://doi.org/10.3390/vetsci9100519>
- Travain, T.; Valsecchi, P. Infrared Thermography in the Study of Animals' Emotional Responses: A Critical Review. *Animals* 2021, 11, 2510. <https://doi.org/10.3390/ani11092510>
- Jerem P, Romero LM. It's cool to be stressed: body surface temperatures track sympathetic nervous system activation during acute stress. *J Exp Biol.* 2023 Oct 15; 226(20): jeb246552. doi: 10.1242/jeb.246552. Epub 2023 Oct 31. PMID: 37767773; PMCID: PMC10629684.
- Glenk, L.M.; Foltin, S. Therapy Dog Welfare Revisited: A Review of the Literature. *Vet. Sci.* 2021, 8, 226. <https://doi.org/10.3390/vetsci8100226>
- Budny-Walczak, A.; Wilk, M.; Kupeczynski, R. Infrared Thermography in Assessment of Facial Temperature of Racing Sighthound-Type Dogs in Different Environmental Conditions. *Animals* 2024, 14, 1180. <https://doi.org/10.3390/ani14081180>
- Aragona, F.; Rizzo, M.; Arfuso, F.; Acri, G.; Fazio, F.; Piccione, G.; Giannetto, C. Eye Temperature Measured with Infrared Thermography to Assess Stress Responses to Road Transport in Horses. *Animals* 2024, 14, 1877. <https://doi.org/10.3390/ani14131877>

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Impacts of Animal Assisted Interventions on Elderly Wellbeing and Canine Welfare: A Multiparametric Assessment

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Animal-Assisted Interventions (AAIs) are increasingly used in healthcare settings as an innovative tool to improve elderly wellbeing. This study evaluates the impact of an Animal-Assisted Education project (within the In-Habit H2020 project GA n. 869227) on quality of life, sleep patterns, and stress markers in elderly people residents in two nursing homes, while also assessing the welfare of involved dogs and the possible correlations of endocrine parameters between dogs and their handlers.

Nineteen elderly participants (mean age 81.7 years old, 14 female) and nine dog-handler dyads (dogs' mean age 7.4 years old, 6 female 55.5% mixed breed; handlers' mean age 52.6 all female) were included and monitored at baseline, mid-study, and study completion, in all cases before and after the session. For elderly, quality of life and sleep were assessed through validated questionnaires (Psychological General Wellbeing Index; Pittsburgh Sleep Quality Index) and wearable devices (smartwatch Fitbit ® Alta); salivary cortisol and oxytocin concentrations were measured in elderly as well as in dogs' handlers. Canine welfare was monitored through physiological (heart rate, blood pressure, respiratory rate, rectal temperature), and endocrine parameters (salivary cortisol and oxytocin concentrations).

Statistical analysis comprised descriptive statistics, Wilcoxon rank test, and Friedman test, with a significance level set at $p < 0.05$.

Results in elders indicated a significant improvement in perceived quality of life ($p=0.029$) and sleep duration (Total Sleep Time) ($p=0.009$), although sleep efficiency decreased ($p=0.017$). Salivary cortisol levels significantly decreased in elderly participants after sessions ($p=0.004$ at project end) and through the project ($p=0.023$ from baseline to mid-study). Dogs also exhibited a significant reduction in salivary cortisol levels post-session ($p=0.011$ at mid-study) and through the project ($p=0.028$ from baseline to mid-study), while physiological parameters remained stable after sessions. Handlers' cortisol concentrations significantly decreased post-session ($p=0.011$ at follow-up; $p=0.043$ at project end). No significant correlation was found between handlers and dogs cortisol levels.

These findings suggest that AAIs can enhance psychological wellbeing and sleep quality in elderly participants, while ensuring the welfare of dogs. The reduction in cortisol levels in elderly participants, dogs and their handlers indicates a positive stress-mitigating effect of the intervention. The stable physiological parameters in dogs further suggest that AAIs did not induce stress in the canine participants. The lack of physiological synchrony between handlers and their dogs might be due to the small sample size. Future studies should explore larger, gender-balanced samples and additional endocrine methods for assessing wellbeing dynamics in human-animal interactions.

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Smart textiles to assess physiological and emotional states in horses during Positive Reinforcement Training

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Few studies have reported the effects of Positive Reinforcement Training (PRT) on the physiological/emotional states of horses. Heart Rate Variability (HRV) is considered a measure of the emotional state in animals [1], and in horses, HRV has been extrapolated from ECGs recorded using smart textile technology [2, 3]. This study aimed to determine the physiological/emotional states of horses during PRT using three HRV parameters, namely RMSSD (root mean square of successive RR interval differences, ms), LF/HF (ratio of LF to HF power) and SampEn (sample entropy), detected by smart textiles.

Thirty-four healthy horses were trained to touch a target using PRT. Training sessions lasted from 5 to 15 minutes. The time the horses spent looking at the target/trainer was recorded and converted into percentages of the total training time. Baseline and training session(s) ECGs were recorded using smart textiles and the HRV parameters were extrapolated. Changes in HRV parameters between baseline and training were calculated, with values > 100 and < 100 indicating an increase and decrease in HRV values during training, respectively. Wilcoxon rank-sum test and Spearman's correlation were used to test for differences in changes in HRV parameters in horses that spent more time looking at the target/trainer and the relationship between the time the horses spent looking at the target/trainer and the changes in HRV parameters (significant p -value < 0.05).

85 % of the horses (29/34) looked more at the target and 15% of the horses (5/34) looked more at the trainer, with changes in HRV parameters of 161 ± 30 and 75 ± 13 (mean \pm SEM) for RMSSD, 210 ± 43 and 242 ± 100 (mean \pm SEM) for LF/HF, and 102 ± 8 and 146 ± 26 (mean \pm SEM) for SampEn, respectively. No significant differences were found in the changes in HRV parameters between horses that spent more time looking at the target/trainer. A moderately positive significant correlation was found between the time the horses spent looking at the trainer and the change in SampEn ($\rho = 0.40$, $p = 0.019$), with SampEn increasing the longer the horses looked at the trainer. Our results suggest that individual horses may have felt calmer (i.e., had higher SampEn) during training when looking at the trainer, in line with the literature [4, 5]. This is the first study to use smart textiles during PRT in horses to assess their physiological/emotional state.

[1] E. von Borell, J. Langbein, G. Després, S. Hansen, C. Leterrier, J. Marchant, R. Marchant-Forde, M. Minero, E. Mohr, A. Prunier, D. Valance, and I. Veissier, “Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals — A review”, *Physiol. Behav.*, vol. 92, pp. 293-316, 2007.

[2] C. Scopa, E. Palagi, C. Sighieri, and P. Baragli, “Physiological outcomes of calming behaviors support the resilience hypothesis in horses”, *Sci. Rep.*, vol. 8, pp. 1-9, 2018.

[3] M. Felici, A. R. Reddon, V. Maglieri, A. Lanatà, P. Baragli, “Heart and brain: Change in cardiac entropy is related to lateralised visual inspection in horses”, *PLoS ONE*, vol. 18, pp. 1-8, 2023.

[4] C. Sankey, M.-A. Richard-Yris, S. Henry, C. Fureix, F. Nassur, and M. Hausberger, “Reinforcement as a mediator of the perception of humans by horses (*Equus caballus*)”, *Anim. Cogn.*, vol. 13, pp. 753-764, 2010.

[5] S. Briefer Freymond, E. F. Briefer, A. Zollinger, Y. Gindrat-von Allmen, C. Wyss, and I. Bachmann, “Behaviour of horses in a judgment bias test associated with positive or negative reinforcement”, *Appl. Anim. Behav. Sci.*, vol. 158, pp. 34-45, 2014.

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Body Condition Assessment in Sled Dogs: Intra- and Inter- Observer Agreement of BCS and Its Correlation with Ultrasonography and Plicometry

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Body condition is an important indicator of a dog's energy balance, affecting its performance and well-being, especially for athletic dogs like sled dogs. Proper body condition is essential for maintaining health, and meeting the high metabolic demands of intense exercise, such as the activity endured by sled dogs engaged in prolonged training and long-distance competitions (Figure 1). An effective method to assess a dog's ideal weight is by combining the Body Condition Score



Figure 1 – Sled Dog competition, captured by Dr. Lucia Ramera

(BCS) with direct weight measurements [1]. This study aimed to validate the BCS system and investigate its correlation with objective measurements obtained through ultrasonography and plicometry in 27 Siberian Huskies (16 males, 11 females, aged 1–12 years, mean: 5) from three Italian sled dog teams. BCS evaluations were conducted using a 9-point scale by five independent evaluators in a double-blind procedure. BW (body weight) and anthropometric parameters were measured, including height at withers, chest girth, pelvic circumference, hock-to-stifle length, occipital-to-tail length, skull length and circum-

ference, carpal pad-olecranon distance, tarsal pad-

heel distance. The following anthropometric values were calculated: BMI (body mass index) and %BF (body fat) [2]. Subcutaneous fat thickness was measured using ultrasonography on 4 (chest, flank, thigh, and lumbar) and plicometry on 5 anatomical sites (thorax, flank, lumbar, chest, and stifle) [3]. Statistical tests, including Krippendorff's alpha ($K\alpha$) and generalized linear models, were used to assess BCS reliability, validity, and its associations with other measurements. Analyses were performed using SPSS 25.0, with significance set at $p \leq 0.05$. Intra-observer agreement for BCS values was substantial ($K\alpha=0.734$), while inter-observer agreement was moderate ($K\alpha=0.564$). BCS showed a positive association with chest girth ($p=0.025$), BMI ($p=0.014$), and plicometry of the chest ($p=0.003$) and stifle ($p=0.036$). A negative association was found between BCS and breast plicometry ($p=0.017$). The strongest correlation with BCS was found in the anthropometric measurements obtained through plicometry ($p<0.001$), followed by BMI and stifle ($p=0.018$). Ultrasonography did not show a significant relationship with BCS. These findings support the validity and reliability of BCS as an assessment tool for sled dogs, with significant associations found between BCS and plicometry. Further research is needed to refine objective measurement techniques and explore their impact on sled dog welfare and performance.

[1] International Sled Dog Veterinary Medical Association “Body Condition Score Guidelines” 2021.

[2] Mawby et al. “Comparison of various methods for estimating body fat in dogs,” *J.Am.Anim.Hosp.Assoc.*, 2004.

[3] Payan-Carreira et al. “In vivo assessment of subcutaneous fat in dogs by real-time ultrasonography and image analysis” *Acta Vet.Scand.*, 2016.

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Recognition of horse behaviours using deep learning techniques

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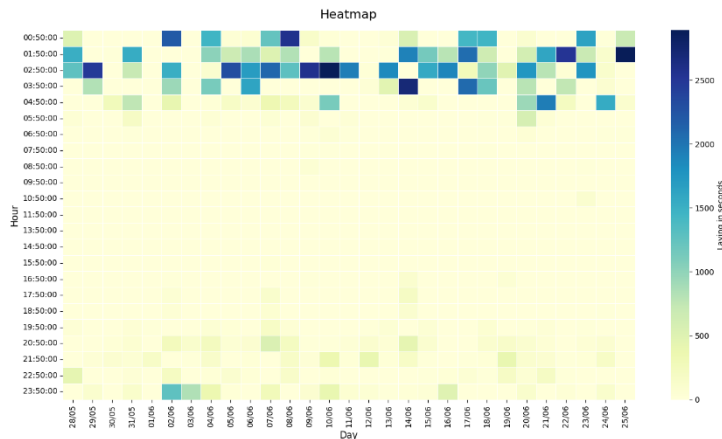


Figure 1 – Heatmap of the time spent lying down from May 28 to June 25 predicted by our computer vision model

The observation of horse behaviour offers important understanding into their state, making it a key indicator of their welfare. Among these behaviours, sleep is particularly important due to its critical biological role in recovery and its cognitive function in memory consolidation [1]. Assessing lying behaviour is an essential aspect of equine welfare evaluation, as horses generally tend to sleep shortly after lying down [3]. As prey animals, horses typically spend between 4 to 15 hours per day to standing rest, while the time spent lying down can range from minutes to several hours [2]. On average, adult horses spent about 80% of their resting time standing [4]. Moreover, monitoring additional behaviours such as access to drinking water and feeding time can provide further information into their welfare. However, directly observing these behaviours, whether in per-

son or via video recordings, can be time-consuming, especially since horses spend only a small portion of their day lying down or drinking. To improve daily management, computer vision technology offers automated methods to interpret and analyze visual data in animal environments [5]. Utilizing methods from image processing and machine learning, computer vision can extract meaningful data and improve the understanding of animal behaviours.

This study investigates the use of a deep learning-based computer vision system to identify the behaviours of individual stabled horses. The initial step involved fine-tuning a pre-trained YOLO architecture to recognize specific behaviours, such as lying, active standing, non-active standing, and drinking, for a single horse housed in a closed box. Object detection methods were used to identify lying and standing behaviours, while pose estimation techniques were utilized to detect drinking activity. To differentiate between active and non-active standing, a pixel-based threshold was applied. The system was then utilized for continuous monitoring over one month, generating a 24-hour time budget for the horse.

The performance of the model was evaluated using precision-recall curves and by comparing its behaviour classifications with manual annotations of the same video data. The system demonstrated an 86% accuracy in behaviour identification relative to human labelling. In conclusion, the technology presented in this study allows real-time recognition and provides valuable information on the welfare of monitored animals. The results highlight the potential of this approach for improving the monitoring and understanding of horse behaviour.

- [1] L. Greening and S. McBride, ‘A Review of Equine Sleep: Implications for Equine Welfare’, *Front Vet Sci*, vol. 9, p. 916737, Aug. 2022, doi: 10.3389/fvets.2022.916737.
- [2] E. L. T. Chung, N. H. Khairuddin, T. R. P. T. Azizan, and L. Adamu, ‘Sleeping patterns of horses in selected local horse stables in Malaysia’, *Journal of Veterinary Behavior*, vol. 26, pp. 1–4, Jul. 2018, doi: 10.1016/j.jveb.2018.03.014.
- [3] U. Auer, Z. Kelemen, V. Engl, and F. Jenner, ‘Activity Time Budgets—A Potential Tool to Monitor Equine Welfare?’, *Animals (Basel)*, vol. 11, no. 3, p. 850, Mar. 2021, doi: 10.3390/ani11030850.
- [4] C. DuBois, E. Zakrajsek, D. B. Haley, and K. Merkies, ‘Validation of triaxial accelerometers to measure the lying behaviour of adult domestic horses’, *Animal*, vol. 9, no. 1, pp. 110–114, Jan. 2015, doi: 10.1017/S175173111400247X.
- [5] P. Kumar, S. Luo, and K. Shaukat, ‘A Comprehensive Review of Deep Learning Approaches for Animal Detection on Video Data’, *IJACSA*, vol. 14, no. 11, 2023, doi: 10.14569/IJACSA.2023.01411144.

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Exploring Saanen lactation curves through Wood and Milkbot models

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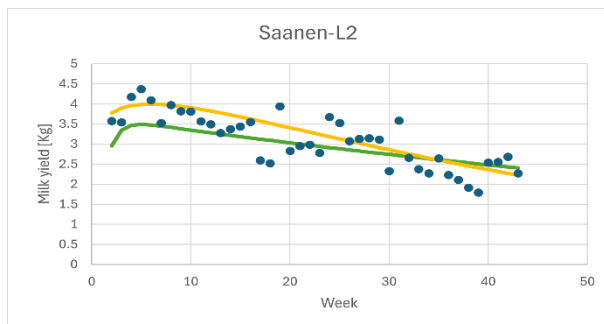


Figure 1. Fitting of Wood and Milkbot equation on Saanen goat data.

Goats milk is known for its healthy quality properties and the lower amount of α s1-casein led to milk goat suitable for children and allergenic people. Unlike dairy cows, the adoption of advanced technologies, such as automatic milking systems, remains limited in goat herds, particularly in rural areas where goats are commonly raised. Milk yields are recorded by hand, making it time-consuming and error prone. As a result, few test day are available in goat lactations.

The analysis of lactation curve shape has been shown to be useful for technicians and farmers to understand the evolution of milk yields and support management decisions. Lactation curve models have not yet been explored in goats opposed to dairy cows where lactation curve model have been adopted by

the entire industry. The aim of our work was delivery a more analytic approach in goats breeding comparing the performance of two mathematical equations - Wood (1) and Milkbot (2) - in fitting the lactation curves shape of Saanen goats. The Wood equation is based on an incomplete gamma function and comprises three key parameters: scale (a), ramp (b), and declining slope (c). The MilkBot model, on the other hand, is an exponential equation and incorporates an additional parameter (decay, d) compared to Wood, enhancing its ability to capture the slope of lactation.

$$Y(t) = at^b e^{-ct} \quad (1)$$

$$Y(t) = a \left(1 - \frac{e^{-\frac{c-t}{b}}}{2} \right) e^{-dt} \quad (2)$$

The analysis was performed on 1024 observations from 248 Saanen goats with parity order equal two and reared in three herds in Southern Italy. The available data contain 19 numeric variables referring to 1) milk yield, 2) milk quality and 3) milk coagulation. The preprocessing analysis was performed in R version 4.1.2. Performance of the final models was evaluated using the coefficient of determination (R^2 (3)).

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}} \quad (3)$$

Wood's model performed slightly better on Saanen with $R^2 = 0.60$, compared to Milkbot with $R^2 = 0.52$, illustrating the ability of two models to fit goats daily milk production. These results encourage adopting a more analytical approaches to goat to obtain in-depth phenotypes on their milk productive capacity. Although, Milkbot model performed slightly worse, it directly provides information on the loss of productivity capacity which can be converted into a measure of persistency. In conclusion, the final aim was promoting the use of more analytic approach in goats breeding.

Use of machine learning models for detection of dry off intramammary infections in dairy cows

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Antimicrobial resistance is a global health threat and creates the urgency to reduce antimicrobial use (AMU) [1]. Dry cow therapy (DCT) is a major contributor to AMU, and implementing selective treatment at dry-off can therefore significantly reduce AMU on dairy farms. Selective dry cow therapy (SDCT) can be applied effectively without increasing the risk of intramammary infections (IMI) during the dry-off period, but its success depends on accurately identifying infected cows [2-3]. Selection methods such as algorithms based on farm data (e.g., somatic cell count (SCC), mastitis history, cow age), show weak correlations with IMI status and while bacteriological analysis is more accurate, it is also costly and labor-intensive [4-5]. Improving selection accuracy is essential to optimize SDCT outcomes, reduce AMU, and ensure animal welfare. The aim of the study was to test the performance of machine learning (ML) algorithms in classifying IMI status at dry-off using data available at the farm level, including both traditional farm data and sensor-derived features. We utilized Dairy Herd Improvement data collected at recurring intervals (approximately every 35 days), including SCC, fat and protein levels and milk yield. Additional data sources included farm records (mastitis history and parity), collar sensors data (rumination and feeding time), and milking parlour sensor data (average milk flux, peak milk flux, and the percentage of time at low milk flux). Bacteriological quarter milk samples were collected from 310 cows before drying off to determine IMI status. We developed and trained six supervised ML models—logistic regression, decision tree, random forest, light gradient-boosting machine (LightGBM), naive Bayes, and neural networks—to predict the infection status of cows (infected or not infected). To comprehensively assess model performance, we evaluated algorithms with all data and excluding sensor data to determine the impact of sensor-derived features on classification accuracy. In our preliminary results, accuracy ranged from 53% to 71%, with the best-performing algorithm using sensor data being Random Forest (69% accuracy), while the best-performing algorithm without sensor data was LightGBM (71% accuracy). The traditional algorithm tested on our dataset achieved an accuracy of 58%. Despite marginal improvement compared to the traditional algorithm, ML models show potential for enhancing IMI detection in SDCT using farm data and sensor-derived features. While bacteriological analysis remains the gold standard, ML-based approaches could offer an improved alternative to traditional selection methods. With further refinement, these models may become valuable tools for reducing AMU while maintaining udder health.

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[1] A. Aidara-Kane, F. J. Angulo, J. M. Conly, Y. Minato, E. K. Silbergeld, S. A. McEwen, P. J. Collignon, and WHO Guideline Development Group. “World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals”, *Antimicrob Resist In*, vol. 7, pp. 7. 2018.

[2] A. Bradley, S. De Vlieghe, M. Farre, L. M. Jimenez, T. Peters, E. S. de Leemput, and T. van Werven. “Pan-European agreement on dry cow therapy”, *Vet Rec*, vol. 182, pp. 637–637. 2018.

[3] F. Kabera, J.-P. Roy, M. Afifi, S. Godden, H. Stryhn, J. Sanchez, and S. Dufour. “Comparing Blanket vs. Selective Dry Cow Treatment Approaches for Elimination and Prevention of Intramammary Infections During the Dry Period: A Systematic Review and Meta-Analysis”, *Front Vet Sci*, vol. 8, pp. 688450. 2021.

[4] Z. Lipkens, S. Piepers, A. De Visscher, and S. De Vlieghe. “Evaluation of test-day milk somatic cell count information to predict intramammary infection with major pathogens in dairy cattle at drying off”, *J Dairy Sci*, vol. 102, pp. 4309–4321. 2019.

[5] S. M. Rowe, A. K. Vasquez, S. M. Godden, D. V. Nydam, E. Royster, J. Timmerman, and M. Boyle. “Evaluation of 4 predictive algorithms for intramammary infection status in late-lactation cows”, *J Dairy Sci*, vol. 104, pp. 11035–11046. 2021.

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Assessing the future production level of dairy cows with machine learning tools

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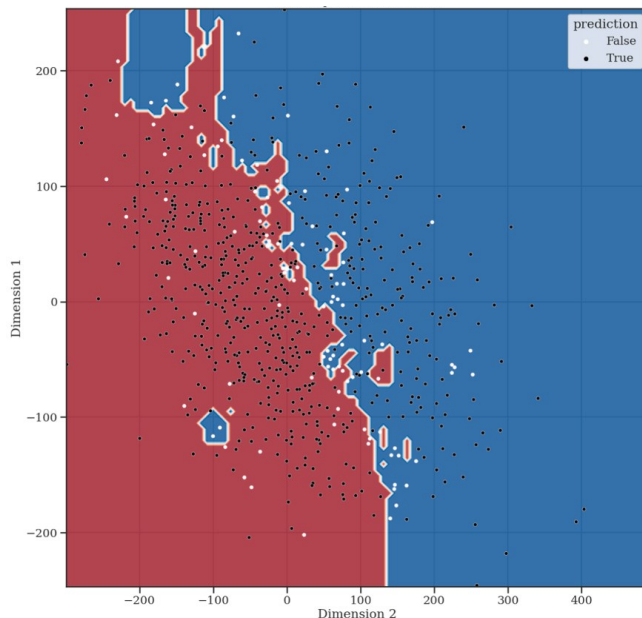


Figure 1 – Classification plot of the lactation curves in a 2-dimension space for the KNN method.

because as is well known, the first lactation usually has a lower production compared to subsequent lactations [4], and for a farmer it is important to know, as soon as possible if, compared to the other animals in the herd, a specific animal will have, on a long term, high, medium or low milk productivity [5]. In the study, firstly two supervised learning methods, i.e., Super Vector Machine and K-Nearest Neighbors, have been applied to a large dataset of 720 complete lactations, with the object to train machine learning tools for the classification between first and second lactation (see Figure 1). Then, for those cows having available the data of first and second lactation curve, the two classification methods have been trained and tested for the attribution of the second lactation productivity level (i.e., low, medium or high) starting from the data of the first lactation. The classification methods reached accuracy values ranging from 70% to 73%. These values seem very encouraging and indicate that the predictors selected, despite their simplicity, look very promising and could pave the way for the definition of enhanced future models.

The challenges of the sustainability in the dairy cattle sector involve milk yield and milk quality levels, cow health and wellbeing, efficient resource use, and emissions reduction. To this regard, in the recent years, several steps forward have been made to increase the production per lactation of the individual animal by working on the genetic selection, on feeding, increasing animal welfare and the quality of the housing environment. Many of these actions are related to daily management decisions that the farmer, nowadays, can undertake with the help of commercial management tools or decision-support systems often associated with sensors or technologies that allow real-time monitoring of the production and health status of the individual animal [1]. Furthermore, the growing widespread of automatic milking systems and electronics milking parlours provide farmers and technicians with continuous series of de-tailed data useful to assess health conditions and evaluate parameters connected to the milk quality and quantity [2]. But, while most of the recent studies investigated models primarily focusing on the prediction of the daily milk yield for the running lactation period one of the still open matters involves the prediction of cow productivity in future lactation periods [3]. This aspect is particularly important in the first years of life of cows, be-

- [1] Giannone, C., Bovo, M., Ceccarelli, M., Torreggiani, D., & Tassinari, P. (2023). Re-view of the Heat Stress-Induced Responses in Dairy Cattle. *Animals*, 13(22). <https://doi.org/10.3390/ani13223451>.
- [2] Ozella, L. et al. (2023) 'A Literature Review of Modeling Approaches Applied to Data Collected in Automatic Milking Systems', *Animals*, 13(12). Available at: <https://doi.org/10.3390/ani13121916>.
- [3] Rebuli, K.B. et al. (2023) 'Multi-algorithm clustering analysis for characterizing cow productivity on automatic milking systems over lactation periods', *Computers and Electronics in Agriculture*, 211, p. 108002. Available at: <https://doi.org/10.1016/j.compag.2023.108002>.
- [4] Masia, F.M. et al. (2020) 'Modeling variability of the lactation curves of cows in auto-mated milking systems', *Journal of Dairy Science*, 103(9), pp. 8189–8196. Avail-able at: <https://doi.org/https://doi.org/10.3168/jds.2019-17962>.
- [5] Arulnathan, V. et al. (2020) 'Farm-level decision support tools: A review of methodo-logical choices and their consistency with principles of sustainability assessment', *Journal of Cleaner Production*, 256, p. 120410. Available at: <https://doi.org/https://doi.org/10.1016/j.jclepro.2020.120410>.

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Real-time classification of ovine pulmonary diseases in sheep lungs using AI-powered CNN models and edge computing

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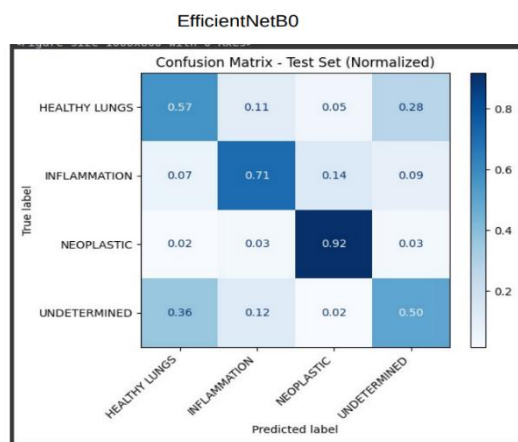


Figure 1 - Diagnostic performance of EfficientNetB0 for the detection of sheep lung pathologies across four different categories.

A total of 1160 JPEG images with different augmentation techniques such as rotation, zoom, and flipping were analyzed using four convolutional neural networks (CNNs) - EfficientNetB0, VGG16, VGG19, and ResNet50, - to classify lung conditions into four categories: healthy, inflammation, neoplastic, and macroscopically undetermined.

Among the tested models, EfficientNetB0 demonstrated superior classification performance, achieving better precision, recall, and F1-score compared to the other architectures, demonstrating its robust ability to detect and classify lung conditions. While the model shows promising performance across most categories, further improvements - such as corroborating results with histopathological diagnoses and expanding the training dataset to include more diverse samples - are recommended to further refine the model's accuracy, particularly in ambiguous or borderline presentations.

By automating macroscopic lung examinations, this AI-driven solution significantly improves diagnostic efficiency and timeliness by providing a reliable and scalable method for automating the macroscopic detection of lung diseases in sheep, ultimately enhancing animal care and driving improved production performance.

Timely and accurate detection of ovine pulmonary diseases is essential for maintaining healthy flocks and optimizing farm productivity, yet traditional meat inspection methods often struggle with challenges such as human error, time constraints, and limited detection of early-stage infections [1]. Consequently, proactive screening of pulmonary infections at the slaughterhouse provides a precise assessment of lung health, enabling prompt, targeted treatments that limit the spread of infection, reduce animal mortality, enhance production outcomes, and promote animal well-being [2].

In this study, we propose a real-time classification system designed to differentiate between healthy lungs and those affected by inflammatory and neoplastic pulmonary pathologies in sheep. Our approach leverages AI-powered Convolutional Neural Network (CNN) models integrated with edge computing solutions, utilizing devices such as the NVIDIA Jetson Nano and the Raspberry Pi 5 for real-time inference.

[1] P. R. Scott, M. P. Dagleish, and C. Cousens, "Development of superficial lung lesions monitored on farm by serial ultrasonographic examination in sheep with lesions confirmed as ovine pulmonary adenocarcinoma at necropsy," *Irish Vet. J.*, vol. 71, pp. 1–9, 2018.

[2] T. Ninios, J. Lundén, H. Korkeala, and M. Fredriksson-Ahomaa, *Meat Inspection and Control in the Slaughterhouse*. John Wiley & Sons, 2014.

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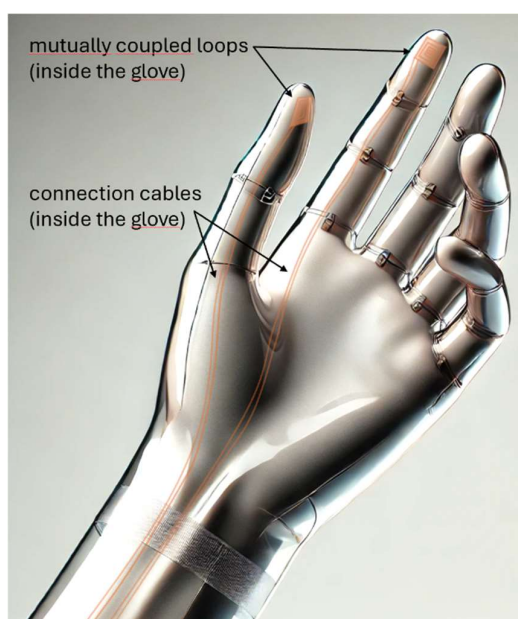
Assessment of Pelvic Diameter in Calves During Parturition Using Measurements of the Leg Distal Bone Diameters to Determine Delivery Method

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The process of parturition in cattle can present significant challenges, particularly when it comes to determining the most appropriate delivery method [1]. Accurate assessment of pelvic diameter is crucial in deciding whether to proceed with a C-section or allow for natural delivery in the cow [2,3]. Traditional methods of pelvic measurement it's not doable at the end of gestation. This study proposes a non-invasive approach using measurements of the calf leg distal bone diameters to predict pelvic diameter and guide delivery decisions.

The ultimate aim of the work presented in this study is to evaluate the effectiveness of using calf carpus measurements during calving to assess pelvic diameter and make informed decisions on whether to proceed with a caesarean or natural delivery. To do this, this work presents the study and prototype realisation of a sensorised glove, equipped with two mutually coupled copper coils of an appropriate shape positioned on the fingertip of the index finger and thumb finger of the hand as shown in Figure 1. By measuring the mutual coupling of the coils, it is possible to estimate the reciprocal distance of the central points of the coils themselves and thus indirectly derive the diameter of the object enclosed between the thumb and index finger of the hand (i.e. the calf carpus). In the first part of the paper, we study through

the method of partial mutual inductances the mutual coupling behaviour as the mutual position of the turns changes, including the angle and the distance between them, in order to assess the sensitivity of the proposed method and to optimise the shape of the turns adopted.

Once the theoretical study was completed, a laboratory prototype was realised to verify through measurements the effectiveness of what was proposed. A series of diameter measurements of known objects was carried out and compared with the diameter estimate obtained from the sensorised glove, to assess its reliability and effectiveness.

Future developments of the work include the field study on a sample of Holstein-Frisian pregnant cows in a controlled environment, measuring the carpus diameter of calves at different stages of parturition using the proposed system and subsequently measuring the corresponding pelvic diameters. An analysis of the data will be performed to identify correlations between leg distal bone and pelvic diameters, respectively, in order to establish a threshold carpal diameter for predicting the need for a caesarean section.

- [1] A. Naazie, M. M. Makarechian, R. T. Berg – “Genetic, phenotypic, and environmental parameter estimates of calving difficulty, weight, and measures of pelvic size in beef heifers” - Journal of Animal Science, Volume 69, Issue 12, December 1991, Pages 4793–4800;
- [2] Z. Nogalski – “Relations between the course of parturition, body weights and measurements of Holstein-Friesian calves” Czech Journal Anim. Sci., Volume 48, Year 2003 (2), Pages 51–59;
- [3] A. Naazie, M. Makarechian, R. T. Berg – “Factors Influencing Calving Difficulty in Beef Heifers” - Journal of Animal Science, Volume 67, Issue 12, December 1989, Pages 3243–3249;

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Performing Pregnancy Tests Milk and Their Effect on Cow Welfare Compared to Rectal Pregnancy Tests 40 to 45 Days Post Insemination

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The main pregnancy test method for cows is rectal palpation. Rectal palpation requires cow's restriction in headlocks for hours and may result in physiological and behavioural changes that are typical of a stress response [1, 2]. Today, the absence of stress responses is accepted as an indicator of welfare in animals. The rectal method is a tied invasive examination that disrupts the cow's routine, potentially affecting its well-being and welfare [3, 4]. The objective of this study was to evaluate whether a milk-based pregnancy test improves cow welfare compared to rectal palpation in dairy farms. We use assays for detecting pregnancy-associated glycoprotein (PAG) on milk originating from the embryonic trophoblast by using a quantitative milk Elisa [Milk Pregnancy Test, IDEXX]. The effects on cow welfare were assessed by the use of pedometers [neck & leg] and by use of new collecting data sensors and software [Afimilk, Afikim, Israel]. The study was done in two large intensive dairy farms [n=1,517]. Cows were divided into two groups, each tested on the same day (42-45 post insemination) using either the milk-based or rectal palpation method. Tested parameters were measured between two consecutive milking [8 hours]. Tested parameters were: 1. Number of times locking up the cows for the rectal examination, 2. Length of time from tying to rectal examination by the veterinarian 3. Total time that the cow was isolated from its group. We found that the results using pregnancy milk test got **less**: Cow/ Steps/ Hour [$p \leq 0.05$], less Time out of their group [4-5 h,] and less tied Time [4-6 h] in the other hand we found increase in cow rest time [90 min. $p \leq 0.001$]

Conclusion: Use of milk test pregnancy prevent unnecessary restriction and improve the cow welfare by increasing the rest time. In addition use of this method will provide the practitioner more time to deal with real professional problems on the farm.

[1] S. Waiblinger, C. Menke, J. Korff, A. Bucher. "Previous handling and gentle interactions affect behaviour and heart rate of dairy Cows during a veterinary procedure". *Appl. Anim. Behav. Sci.*, 85, 31–42, 2004.

[2] T. Nakao, T. Sato, M. Moriyoshi, K. Kawata. "Plasma Cortisol response in dairy cows to vaginoscopy, genital palpation per Rectum and artificial insemination". *J. Vet. Ser. Med. A*, 41, 16–21, 1994.

[3] C. Cagri Cingi, D. Fatih Baser, Y. Sunucu Karafakioglu, A. Fati Fidan. "Stress response in dairy cows related to rectal Examination". *Acta Sci. Vet* 40, 1053. 2012.

[4] H. Giese *et al.* "Influence of trans rectal palpation training on cortisol levels and heart rate variability in cows", *The-riogenology* pp. 238–244, 2018.

Evaluation of a New Technological Device for Continuous Monitoring of Vital Functions in Newborn Foals

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The use of technological devices capable of continuously monitoring vital parameters in newborn foals could be extremely useful for the early identification of neonatal pathologies. Currently, there are no technological devices available for these species. This study aimed to validate a multiparametric wearable device developed for telemonitoring of large dogs (Dinbeat UNO®, Dindog Tech, Spain) by adapting it to nine newborn foals. The following eligibility criteria were applied to enroll the animals: eutocic birth, Apgar score > 9 and healthy clinical examination two hours after birth. The device measured heart rate (HR), body temperature (T°C) and respiratory rate (RR); in addition, an electrocardiogram (presence/absence of arrhythmias) and the different positions (standing/lying) and activities (resting/walking) of the animals were recorded. It was applied 8 hours after birth and recorded data for 6 hours. Gold standard measurements included body temperature assessment using a digital thermometer, heart rate assessment using direct auscultation, arrhythmia detection using a portable electrocardiograph, RR, position and activity monitoring. Each parameter was assessed every 10 minutes during wearable device recording.

Inter-rater reliability between device and veterinarian was assessed using an intraclass correlation coefficient (ICC) or Cohen's kappa (K) for continuous and categorical parameters, respectively. Correlation between continuous variables was assessed using Pearson's test (r), while for categorical variables, Spearman's test (rs) was used.

During the recording process, 344 pairwise determinations were compared for the continuous variable RR, 326 for HR and 448 for T°C. For categorical variables, measurements included 448 for position parameter, 438 for activity and 300 for "presence/absence" of arrhythmias. The inter-rater reliability between the device and the gold standard was excellent for position detection and arrhythmia detection (K=1), good for RR (ICC=0.801) and HR (ICC=0.852), as well as for activity (K=0.898), while it was poor for T°C (ICC=0.346). Regarding the correlation between continuous variables, RR showed a moderate correlation (r=0.700; P<0.0001), while T°C showed a weak correlation (r=0.371; P<0.0001). For all categorical variables, a complete agreement between the device and the gold standard was found (rs=1.000 - very strong, P=0.01). Although most of the evaluated parameters showed a concordance between the device and the gold standard, the use of a device adapted from another species may have influenced the recording of some parameters, in particular T°C. Overall, the device appears to provide valuable predictive information on the vital parameters of the subjects. However, the development of species-specific devices to monitor the health status of newborn foals remains a priority.

[1] Woort F et al. Validation of an equine fitness tracker: ECG quality and arrhythmia detection. *Equine Vet J.*, 55(2):336–43, 2023.

Hormonal Changes as Physiological Indicators of Welfare in Rabbit does Supplemented with Omega-3

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Omega-3 fatty acids have the potential to enhance animal welfare by modulating inflammation, improving metabolism, and supporting reproductive and immune functions [1]. This study investigated the effects of dietary omega-3 supplementation with extruded linseed and a combination of extruded linseed and *Padina pavonica* algae on metabolic hormones and metabolites in female rabbits over one reproductive cycle, emphasizing the role of hormone monitoring as a key indicator of animal welfare. Forty nulliparous female rabbits were divided into three groups (n=13) and fed different diets: control (CNT), L5% (5% extruded linseed), and L5%PP (5% extruded linseed + 0.2% *Padina pavonica* extract). Blood samples were collected at five time points: at the beginning of the experiment (T0), artificial insemination (T1), kindling (T2), end of lactation (T3), and weaning of the rabbit kits (T4). Cortisol, insulin, leptin, glucose, and non-esterified fatty acids (NEFA) were analyzed using radioimmunoassay (RIA) and enzymatic colorimetric methods [2]. Data were analyzed using Linear Mixed Models. Cortisol levels peaked at kindling in all groups (p<0.001), reflecting the physiological increase associated with parturition. Insulin levels were significantly influenced by dietary treatment (p=0.016), with the L5% group showing higher marginal means compared to CNT (p<0.05). At the end of lactation, the CNT group had the lowest insulin values (p=0.05), while at weaning, the LPP group exhibited lower values than the L5% group (p<0.05). These findings support the established potential of linseed and *Padina pavonica* to modulate insulin metabolism, likely through distinct mechanisms that influence insulin sensitivity [3]. Leptin was consistently higher than baseline (T0) at all time points (p<0.05). A significant difference was observed at weaning, with CNT group showing higher leptin levels (p<0.05), suggesting that linseed and algae supplementation may have more efficiently regulated fat accumulation, potentially resulting in a more favourable metabolic profile and reduced excessive fat deposition [2]. Glucose concentrations decreased over time (p<0.001) without significant differences among groups, whereas NEFA levels increased at kindling, end of lactation, and weaning (p<0.001), indicating the physiological metabolic adaptations to support milk production. In conclusion, monitoring hormonal levels is crucial for assessing animals' metabolic status, directly affecting health, fertility, and well-being. Understanding these hormonal dynamics can guide the development of dietary strategies to improve welfare and productivity, ultimately promoting more sustainable and efficient rabbit farming practices.

[1] S. Agradi et al. "Dietary supplementation with n-3 polyunsaturated fatty acids: Effects on reproductive and productive performance and meat quality in rabbit breeding" *Anim. Nutr.* 2023.

[2] L. Menchetti et al. "Energy homeostasis in rabbit does during pregnancy and pseudopregnancy" *Anim. Reprod. Sci.* 2020.

[3] T.L. Gunathilaka et al. "Antidiabetic potential of marine brown algae – a mini review" *J. Diabetes Res.* 2020.

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Metabolic Response and Growth Performance in Fattening Rabbits Supplemented with Linseed and *Padina Pavonica* Algae

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Dietary supplementation with omega-3 polyunsaturated fatty acids (PUFA) has been shown to positively influence various aspects of animal health [1]. This study aimed to evaluate the metabolic response of fattening rabbits to dietary supplementation with extruded linseed (5%) and a combination of linseed (3.5%) and algae *Padina pavonica* (0.2%). Forty-five New Zealand White growing rabbits, weaned at 35 days of age and slaughtered at 85 days, were divided into three groups (n=15): the control group (CNT) received a standard diet, while the other two groups received modified isoenergetic diets in which part of the CNT diet ingredients were replaced with 5% extruded linseed (L) and 3.5% extruded linseed plus 0.2% *Padina pavonica* algae extract (LPP). Productive performance parameters, including body weight (BW), average daily gain (ADG), and feed conversion ratio (FCR), were assessed weekly, while feed intake (FI) was recorded daily. Blood samples were collected to evaluate the metabolic status at weaning, 60 days, and slaughter to assess insulin, leptin, non-esterified fatty acids (NEFA), and glucose. The results for productive parameters showed that the L group had higher marginal means for BW ($P<0.05$), while no significant differences in FI were observed among groups. Additionally, compared to the LPP group, the L group exhibited a higher ADG and lower FCR ($P<0.05$). These findings suggest that dietary supplementation with marine algae did not negatively affect feed intake, addressing previous concerns regarding its palatability. Moreover, the L group demonstrated better growth performance, characterized by greater weight gain and feed efficiency. Hormonal analysis of the metabolic profile revealed that the LPP group had lower insulin levels compared to the L group at 35 (weaning) and 60 days ($P<0.05$), suggesting that algae supplementation may improve insulin sensitivity [2]. Leptin levels increased with age ($P<0.001$), reflecting greater fat deposition and increased metabolic activity. Glucose levels declined over time ($P<0.001$), likely due to increased utilization for growth. NEFA concentrations decreased particularly at 60 days ($P<0.01$), indicating improved energy efficiency and reduced lipid mobilization. These findings highlight metabolic adaptations in fattening rabbits and suggest that algae and linseed supplementation may impact insulin sensitivity and energy metabolism. In conclusion, monitoring the metabolic profile in fattening rabbits is crucial for understanding the impact of dietary interventions on both health and production, as it provides key insights into the physiological adaptations that influence growth, efficiency, and overall well-being.

[1] Agradi, S. et al. Dietary Supplementation with N-3 Polyunsaturated Fatty Acids: Effects on Reproductive and Productive Performance and Meat Quality in Rabbit Breeding. *Animal Nutrition* 2023

[2] Al-Soufi S, et al. Marine Macroalgae in Rabbit Nutrition-A Valuable Feed in Sustainable Farming. *Animals* (Basel). 2022

Hair steroid quantification: a non-invasive method to assess reproductive and welfare in wild and laboratory species

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The Hypothalamic-Pituitary-Gonadal (HPG) and Hypothalamic-Pituitary-Adrenal (HPA) axis are the main pathways responsible for behavioural modulation and reproduction[1]. In animal studies, steroids have mainly been analysed and quantified on several matrices. However, hair steroids have been studied and analysed in numerous species including domestic animal, carnivorous, rodents, livestock and cervids in wild and laboratory setting [2], [3], [4], [5]. Especially for studies in wildlife and lab species, animal who are difficult to hold or difficult to sampling high quantity [6]. Hair from 4 different species were collected: 28 sexually mature roe deer bucks (*Capreolus capreolus*), 10 pregnant female red deer (*Cervus elaphus*), 20 deceased (11 male and 9 female, young, sub-adult and adult) Appenine wolf (*Canis lupus italicus*) and 56 sexually mature male mice (*Mus musculus*). Briefly, hair was washed with water and propanol-2-ol. Once fully dried, hair was finely pulverized. Each pulverized sample was incubated overnight with methanol for steroids extraction. After centrifugation, methanol was collected and evaporated to dryness under an air-stream suction hood. All steroids quantification was performed with radioimmunoassay (RIA) except for ELISA test in corticosterone in mice. In roe deer testosterone (T) and cortisol (CORT) were quantify during pre- and post-rut period. Statistical differences were founded between periods, with opposite trends (Hair testosterone pre-rut 1.48 pg/mg and post-rut 2.49 pg/mg, hair cortisol pre-rut 8,72 pg/mg and post-rut 0,49 pg/mg). Progesterone (P4) and CORT were evaluated in red deer. The hair CORT and P4 ranges are respectively 0.03–0.55 pg/mg and 19.47–153.57 ng/mg with no statistical differences. Corticosterone and testosterone (T) hormonal hair profile of laboratory male mice were investigated to verify a potential relationship with age and housing. Hormone levels (6.42 pg/mg for T 502.1 pg/mg for corticosterone, 23.16 ± 7.00 pg/mg for dehydroepiandrosterone) showed a statistical correlation between each other, no statistical differences between the two groups (pair and groups) were founded for T and corticosterone. The concentration of DHEA was statistically affected by the type of housing ($p = 0.0377$, Student's t test). These results suggested that such housing practice may be beneficial for social interactions. In the end, in Appennine wolf CORT, T, DHEA, P4, and oestradiol (E2), were assed using radioimmunoassay, but no statistical differences were founded between groups. These results showed to possibility to observe the physiological pathways and animal welfare from analysis from hair in wild or laboratory animals, where it is often difficult to collect other matrices for analysis.

[1] A. Elmi *et al.*, «Comparison of hair steroid levels among Wistar rats exposed to different environmental enrichment settings», *Vet World*, pp. 2731–2735, dic. 2024, doi: 10.14202/vetworld.2024.2731-2735.

[2] A. Elmi *et al.*, «Testicular Melatonin and Its Pathway in Roe Deer Bucks (*Capreolus capreolus*) during Pre- and Post-Rut Periods: Correlation with Testicular Involution», *Animals*, vol. 11, fasc. 7, p. 1874, giu. 2021, doi: 10.3390/ani11071874.

[3] A. Elmi *et al.*, «Quantification of Hair Corticosterone, DHEA and Testosterone as a Potential Tool for Welfare Assessment in Male Laboratory Mice», *Animals*, vol. 10, fasc. 12, p. 2408, dic. 2020, doi: 10.3390/ani10122408.

[4] D. Ventrella *et al.*, «Progesterone and Cortisol Levels in Blood and Hair of Wild Pregnant Red Deer (*Cervus Elaphus*) Hinds», *Animals*, vol. 10, fasc. 1, p. 143, gen. 2020, doi: 10.3390/ani10010143.

[5] L. A. Frank, K. A. Hnilica, e J. W. Oliver, «BlackwellPublishing,Lt Ad. drenal steroid hormone concentrations in dogs with hair cycle arrest (Alopecia X) before and during treatment with melatonin and mitotane», *Veterinary Dermatology*, 2004.

[6] J. Wojtaś, «Hair cortisol levels in cats with and without behavioural problems», *Journal of Feline Medicine and Surgery*, vol. 25, fasc. 2, p. 1098612X221150624, feb. 2023, doi: 10.1177/1098612X221150624.

Animal Welfare Measures in the CAP 2023 - 2027: a Comparison of Member States with a Focus on Italian Outcomes During the First Year of Implementation

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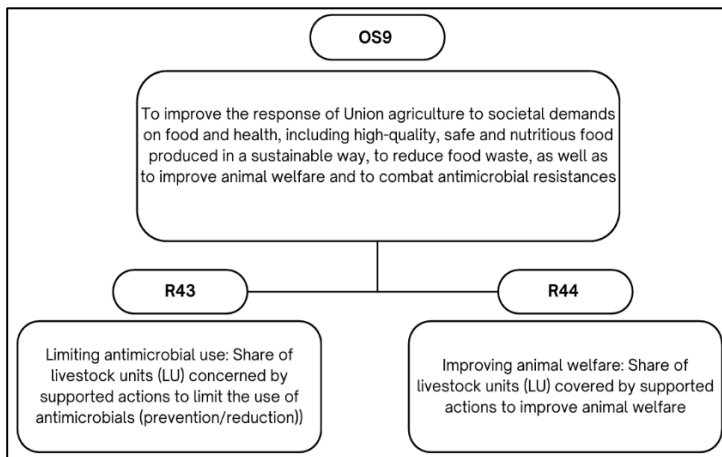


Figure 1 – CAP specific goal and result indicators of animal welfare

Common Agricultural Policy (CAP) instruments and measures have the potential to contribute to animal welfare and antimicrobial use reduction [1]. However, assessing their impact has been difficult due to the lack of specific indicators in previous programming periods (2014–2020). Until recently, no standardized methods existed for defining, collecting, and interpreting indicators to reliably assess animal welfare. Interest in animal welfare has shifted from mitigating negative conditions to ensuring positive animal experiences throughout their lives [2]. For the 2023–2027 period, the European Commission recommended that Member States report the percentage of livestock units affected by measures targeting animal welfare (R44) and antimicrobial reduction (R43) [3].

Evaluating the CAP's impact should rely on specific indicators integrated into the policy framework [4]. The most used indicators in EU projects are grouped into three categories: animal-based, resource-based, and management-based [5]. Recent studies highlight the importance of integrating physiological parameters, such as cortisol levels, heart rate variability, and immune function, into these assessments to enhance accuracy and objectivity. Colditz *et al.* (2013) emphasize that combining physiological measures with traditional indicators improves the reliability of welfare evaluations, particularly in regulatory compliance and funding decisions, by providing scientific data reflecting both physical and psychological well-being [6].

This study compares animal welfare measures in the CAP Strategic Plans of Italy, France, Germany, Spain, and Portugal. Using a comparison matrix, the analysis identifies measures linked to 15 key indicators commonly applied in EU projects to assess animal welfare and antimicrobial use. Focus is placed on Italy, evaluating outcomes of the first year. A first evaluation of the Italian Strategy for Animal Welfare highlights a trend towards strengthening the incentives provided for the reduction of antibiotic resistance (AMR) in line with one of the 15 indicators identified by the Commission. Data from the National Paying Agency (AGEA) [7] and regional agencies show strong breeder participation. In the bovine sector, eligible Livestock Units (LUs) under Ecoscheme 1 “Payment for Antibiotic Resistance and animal welfare” covered 69% of the national cattle population [8], involving 2.8 million LUs in efforts to curb antibiotic resistance. However, the reduction in antimicrobial use is only an initial data point, influenced by factors beyond policy incentives. Mormède *et al.* (2007) highlight that antimicrobial usage variations are linked not only to management practices but also to physiological conditions like stress, immune function, and metabolism [9]. The study's second phase will link key measures to physiological parameters found in the literature, validating welfare outcomes with objective data.

[1] *Européen D*, G., & Economique, I. (2021). *Agrosynergie Study on CAP measures and instruments promoting animal welfare and reduction of antimicrobial use Executive summary*.

[2] *Assessment of result-based interventions Thematic report*. (n.d.). <https://eu-cap-network.ec.europa.eu/support/evaluation>.

[3] Regulation (EU) 2021/2115 of the European Parliament and of the Council of 2 December 2021

[4] European Evaluation Helpdesk (2022) *cap evaluation newsletter*, June 2022

[5] Européen D*, G., & Economique, I. (2021). *Agrosynergie Study on CAP Measures and Instruments Promoting Animal Welfare and Reduction of Antimicrobials Use Final report*.

[6] Colditz, I., Ferguson, D., Collins, T., Matthews, L., & Hemsworth, P. (2013). 2. *Assessing the Welfare of Farm Animals-A Review Animal Welfare Science Centre-Identify and integrate measures of animal welfare that meet the needs of animals and society*. In Matthews & Associates Research International (Vol. 4).

[7] Our elaborations on data's National Paying Agencies (AGEA) and the regional Paying Agencies as of 31.07.2024

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- [8] Nazionale cattle population: 31.12.2023: https://www.vetinfo.it/j6_statistiche/#/report-pbi/11 (accessed: January 6, 2025).
- [9] Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C. G., Richard, S., & Veissier, I. (2007). Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. In *Physiology and Behavior* (Vol. 92, Issue 3, pp. 317–339). Elsevier Inc. <https://doi.org/10.1016/j.physbeh.2006.12.003>

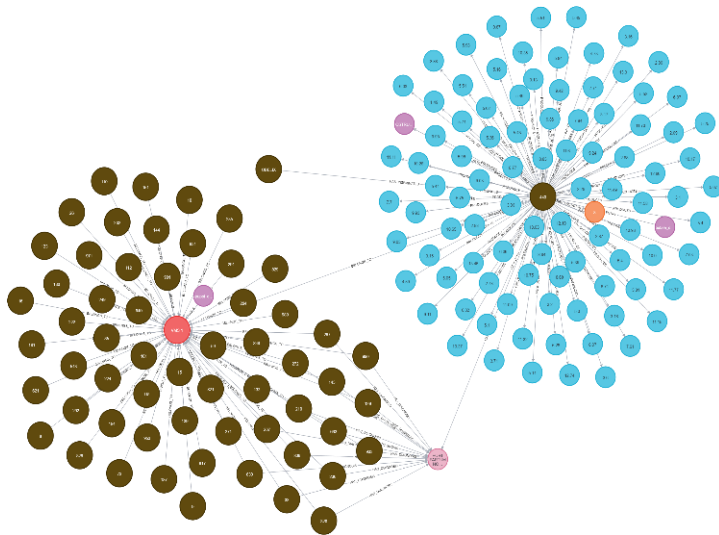
An innovative approach to raise and manage buffalo herds

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Nowadays, it is mandatory the modernization of breeding techniques to enhance production efficiency [1]. Precision Livestock Farming (PLF) employs tools and devices to collect and analyze animal and environmental data [2]. Indeed, advanced analysis, including machine learning and artificial intelligence (AI), is crucial for processing complex datasets and supporting productivity, reproduction and animal welfare in dairy production.

The PLF approach is widely applied in bovine but less commonly used in other

species, such as buffalo [3]. One of the limitations of the advanced PLF techniques is the difficulty of the farmers to adopt them, since they are not able to understand AI processes and interpret results properly.

To this aim, a case study in the buffalo sector was developed by Logogramma, leveraging AI.CODIUM[®] a proprietary platform for the development of human-machine interaction applications.

The aim of the application is not only to gather sensor data into a single platform but also to dynamically integrate human experience and knowledge, thereby increasing the reliability of the collected data while preserving the explainability of the outputs. The availability of such structured and integrated data enables operators to query the knowledge base using natural language, instantly retrieving relevant information in both written and visual formats.

Thanks to the data provided by "Tenuta Vannulo" - one of the first farms adopting automated milking and monitoring systems, thus demonstrating the benefits of a data-driven approach in enhancing animal welfare and production - the application has actually shown suitability for:

- Collecting human observations and integrating them with data from external sources such as farm sensors, cameras, scales, and tags. This enables the tracking of each buffalo's wellness history, group affiliation, insemination records, or human diagnosis.
- Processing natural language requests through a voice assistant, delivering relevant responses based on the collected data stored within the knowledge graph.

- Generating insights using an "Intelligent Analytics" module, which provides visualizations such as charts and graphs representing metrics like the lactation curve and average daily milk production.

All data is, thus, structured into a unified knowledge graph powered by AI.CODIUM®, enabling advanced data analysis and predictive algorithms supported by AI and Deep Learning. Additionally, it can provide companies with actionable suggestions for best practices and potential improvements, delivered in natural language via the app.

- [1] L. Trapanese, M. Hostens, A. Salzano, N. Pasquino, Short review of current limits and challenges of application of machine learning algorithms in the dairy sector, *Acta IMEKO* 13 (2024) 1–7. <https://doi.org/10.21014/actaimeko.v13i1.1725>.
- [2] D. Berckmans, Precision livestock farming technologies for welfare management in intensive livestock systems, *Revue Scientifique et Technique de l'OIE* 33 (2014) 189–196. <https://doi.org/10.20506/rst.33.1.2273>.
- [3] L. Benos, A.C. Tagarakis, G. Dolias, R. Berruto, D. Kateris, D. Bochtis, Machine Learning in Agriculture: A Comprehensive Updated Review, *Sensors* 2021, Vol. 21, Page 3758 21 (2021) 3758. <https://doi.org/10.3390/S21113758>.

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Leveraging Machine Learning and Explainable AI for Animal Health Assessment and Nutritional Analysis

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Machine Learning (ML) has emerged as a transformative tool in animal and veterinary sciences, providing innovative solutions for disease diagnosis, livestock management, and overall animal health monitoring. The ability of ML to analyze large and complex datasets enables veterinarians and researchers to make data-driven decisions, improving both preventive and therapeutic approaches. This study explores how ML techniques are applied in various aspects of veterinary sciences, including disease prediction, precision livestock farming, and behavioral analysis [1].

A Random Forest classifier was trained on a dataset containing physiological parameters, blood biomarkers, movement patterns, and behavioral indicators collected from veterinary records and animal monitoring systems. The dataset included features such as body temperature, heart rate, blood parameters, movement patterns, and behavioral indicators. The RF algorithm was chosen for its ability to handle high-dimensional data, manage missing values, and provide high classification accuracy. The model's performance was evaluated using accuracy, F1-score and AUC metrics [2]. To improve interpretability, SHAP (Shapley Additive Explanations) were applied, allowing veterinarians to understand the contribution of each feature in classification decisions [3].

The RF model effectively analyzed animal characteristics such as age and weight, identifying key predictors related to the impact of different nutrients in their diet. XAI techniques highlighted the influence of specific dietary components on growth patterns and overall health, revealing the importance of nutrient intake on physiological parameters. By integrating XAI, the model provided clear, interpretable insights into how various nutritional factors shape animal development, enhancing the understanding of diet-related effects and supporting more informed decision-making in animal nutrition.

The combination of Random Forest and Explainable AI represents a significant advancement in precision veterinary medicine, offering high accuracy with improved interpretability. The ability to identify key biomarkers and justify predictions enhances model adoption in real-world veterinary practice. Future research should focus on refining XAI methods for real-time disease monitoring, integrating ML models with IoT-based animal health tracking, and expanding datasets to improve model generalization across different species. These findings highlight the potential of ML-driven approaches to revolutionize animal health assessment, disease management, and preventive care strategies.

[1] Akinsulie, Olalekan Chris, et al. "The potential application of artificial intelligence in veterinary clinical practice and biomedical research." *Frontiers in Veterinary Science* 11 (2024): 1347550..

[2] A. Chaudhary, S. Kolhe, R. Kamal, An improved random forest classifier for multi-class classification, *Information Processing in Agriculture* 3 (2016) 215–222.

[3] M. Magarelli, P. Novielli, F. De Filippis, R. Magliulo, P. Di Bitonto, D. Diacono, R. Bellotti, S. Tangaro, Explainable artificial intelligence and microbiome data for food geographical origin: the mozzarella di bufala campana pdo case of study, *Frontiers in Microbiology* 15 (2024) 1393243.

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Exploring Buffalo Lactation Curves with Clustering Algorithms

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A Lactation Curve (LC) is a graphic representation of milk production over one lactation. It follows a characteristic pattern: a peak at the beginning of lactation, representing the maximum milk ejection, followed by a gradual decline. Despite this general trend, variations exist across species, individuals, and parity. By studying LCs, valuable insights can be gained regarding total milk production and lactation persistency, allowing for the selection of the most productive animals. The aim of this work is to group and characterize the LC of buffaloes to investigate physiological differences among clusters and spot potential anomalies in milk production. A preliminary exploratory analysis of buffalo LC was carried out to group and characterize LCs through shape-based clustering. The original dataset contained 661 lactations for 415 animals with a parity order from 1 to 8. To ensure the consistency of lactations, the dataset was divided into 50-day intervals, retaining only those time series that contained at least 70% of the observations within each interval. Lactations were truncated at 270 days in milk and linear interpolation was used to impute missing values. After pre-processing, we obtained a final dataset of 134 lactations. Shape-based clustering was performed, using the k -means algorithm with the Dynamic Time Warping (DTW) distance metric [1], which accounts for variations in the length of the time series by aligning them dynamically. To determine the optimal number of clusters, we employed the Elbow Method, the Silhouette Analysis, and the Davies-Bouldin index. All three methods suggested two clusters.

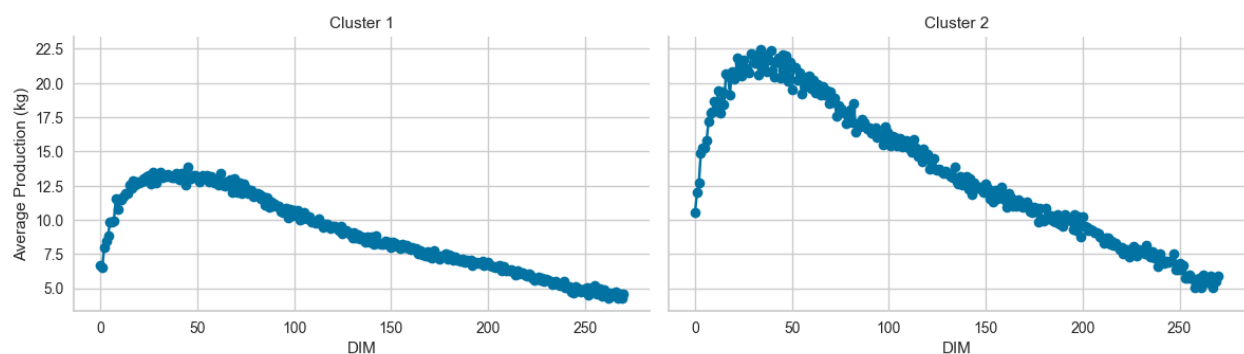


Figure 1. Average Lactation Curves for clusters 1 and 2.

The analysis revealed that Cluster 1 is the largest one, containing 74% of the LCs. In this cluster, the primiparous represented the 93% of all primiparous LCs. A comparison between the two clusters highlighted differences in lactation parameters: buffaloes in Cluster 2 showed higher total average Milk Yield (MY) (3551 ± 674 l vs 2347 ± 454 l, respectively) and average peak MY (30 ± 8 l vs 19 ± 4 l, respectively) compared to Cluster 1. Additionally, the average time to peak in Cluster 2 was lower than in Cluster 1 (37 ± 14 vs 45 ± 23 days, respectively). These findings indicate that one cluster is characterized by higher milk yield and earlier peak production than the other one, making animals grouped in that cluster particularly valuable for breeding selection.

- [1] S. Aghabozorgi, A. Seyed Shirkhorshidi, T. Ying Wah, Time-series clustering – A decade review, Inf Syst 53 (2015) 16–38. <https://doi.org/10.1016/j.is.2015.04.007>.

Assessing Water Scarcity in the Poultry Chain

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Water scarcity has been a critical issue affecting the Mediterranean countries since ancient periods, but, in the last decades, it has increased due to the effects of climate change and population growth. Agriculture and breeding activities are responsible for the highest water requirements due to the high amount needed during the overall life cycle. This paper presents, as the first result of a life cycle assessment (LCA) of the poultry chain, the evaluation of water scarcity focusing on a geographic area of the South of Italy characterized by long periods of drought and high temperatures.

A questionnaire was used for collecting data directly by local companies through the support first of the managers of the Foggia local health authority and then of the staff of the Prevention Department of the Local Health Authority through which the answers to the questionnaires were received by the farmers who have a contract with some big companies. All data collected refers to May 2021. The LCA and Water Footprint methodology [1–3] was adopted and the water requirement of the phase carried out in the areas investigated was compared to the availability of the same area. As for the impact assessment method of water scarcity in the inventory phase, the water use midpoint indicator AWARE (Available Water Remaining) was used for assessing water scarcity [4]. Data were grouped according to the three different types of poultry farming: “Campese” the most widespread with 73 farms, “Conventional” with 32 farms and “Organic” with 25 farms. For each group average data were considered by including both the first and second periods of growth.

According to the various breeding systems, the use of water varies from almost 1800 m³ per cycle of breeding of the organic to around 3500 m³ per cycle of breeding of the conventional. However, the water consumption per chicken remains around 73 litres. According to the life cycle approach, future analysis will take into account other phases of the supply chain such as feed production, transportation and slaughterhouse. However, these first results allow us to understand the dimension of the water requirement for poultry breeding that could help in the calculation of the carrying capacity of a specific area to face water use of the supply chain according to the local water scarcity condition.

Keywords: Water Scarcity, Poultry Chain, Sustainability, AWARE, Life Cycle Assessment,

- [1] ISO 14040: Environmental management - Life cycle assessment - Principles and framework ISO 14040:2006/Amd 1:2020. Geneva: International Standard Organization. (2021)
- [2] ISO 14044: Environmental management - Life cycle assessment - Requirements and guidelines. ISO 14044:2006/Amd 2:2020. Geneva: International Standard Organization. (2021)
- [3] Hoekstra, A.Y.: Water Footprint Assessment: Evolvement of a New Research Field. *Water Resources Management*. 31, 3061–3081 (2017). <https://doi.org/10.1007/s11269-017-1618-5>
- [4] Boulay, A.-M., Bare, J., Benini, L., Berger, M., Lathuillière, M.J., Manzardo, A., Margni, M., Motoshita, M., Núñez, M., Pastor, A.V., Ridoutt, B., Oki, T., Worbe, S., Pfister, S.: The WULCA consensus characterization model for water scarcity footprints: assessing impacts of water consumption based on available water remaining (AWARE). *Int J Life Cycle Assess.* 23, 368–378 (2018). <https://doi.org/10.1007/s11367-017-1333-8>

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Low-Cost IoT Sensors for Environmental Monitoring in Veterinary Applications

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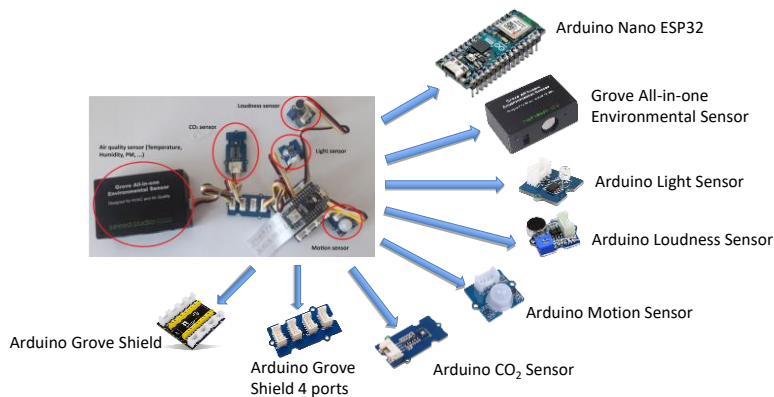


Figure 1 – Arduino based environmental sensor

civil, greenhouse, and agricultural environments [1-3]. By adapting and optimizing these technologies for veterinary applications, we aim to provide a cost-effective and scalable solution for real-time environmental monitoring. This approach complements existing health and feeding management systems, offering a more comprehensive strategy for ensuring optimal living conditions in veterinary settings.

IoT-based sensor networks offer real-time data acquisition and remote monitoring capabilities, allowing veterinarians and farm operators to track critical environmental factors that directly impact animal health, productivity, and biosecurity. Specifically, we investigate the feasibility of using cost-effective sensor platforms based on Arduino and other open-source technologies to measure key environmental parameters such as temperature, humidity, air quality, carbon dioxide and ammonia concentration in livestock facilities.

These systems provide a scalable and accessible solution to enhance early warning mechanisms, optimize resource management, and improve overall animal welfare. This study highlights the benefits and challenges associated with integrating IoT-driven environmental monitoring into veterinary practice. By leveraging low-cost and programmable sensor technology, we demonstrate how real-time environmental data collection can bridge the gap between traditional veterinary care and advanced precision livestock farming. Our contribution is mainly technical, considering the refinement of sensor systems, which, while cost-effective, may not always achieve the same level of precision and accuracy as custom-built devices; and serves as an illustrative example in addressing the challenges of integrating monitoring data into an IoT network. To this aim, we build upon the IoT system developed at the University of Pisa as a model for effectively managing and utilizing sensor data within a connected infrastructure. Based on this network, we analyze specific sensors that can be better suited to veterinary environments, ensuring optimal adaptation to their unique conditions and requirements.

[1] Franco, A., Crisostomi, E., Leccese, F., Mugnani, A., & Suin, S. (2024). Energy Savings in University Buildings: The Potential Role of Smart Monitoring and IoT Technologies. *Sustainability*, 17(1), 111.

[2] Miserocchi, L., Caposciutti, G., Franco, A., & Tellini, B. (2024). Temperature monitoring strategy for microclimate prediction in low-automation greenhouses: a preliminary analysis. In *Journal of Physics: Conference Series* (Vol. 2893, No. 1, p. 012108). IOP Publishing.

[3] Barbaresi, A., Bibbiani, C., Bovo, M., Benni, S., Santolini, E., Tassinari, P., ... & Torreggiani, D. (2020, November). A Smart Monitoring System for Self-sufficient Integrated Multi-Trophic AquaPonic. In *2020 IEEE International Workshop on Metrology for Agriculture and Forestry (MetroAgri-For)* (pp. 175-179). IEEE.

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Adopting insect meal in the Italian trout farming: a multistage process

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The National Plan of the European Maritime, Fishery and Aquaculture Fund (2021-2027) defines three primary challenges facing the aquaculture sector's transformation by 2030: blue transition, digital transition, and resilience. To these is added the intention to support innovation processes transversally to achieve these challenges. The role of innovation as a fundamental component in the shift to new sustainability paradigms has been the subject of extensive discussion [1]. The literature proposed different innovative practices and technologies applicable to aquaculture management, defining them as incremental or disruptive. Novel feeds in fish diets, specifically insect-based feeds, are designated incremental innovations [2] as they do not require substantial organizational modifications [3]. Although compatibility is positively correlated with adoption [4], few authors (e.g., [5]) described it as a semi-incremental innovation since its adoption in aquaculture farms still has drawbacks [6]. Among the overall context-related factors that may influence the adoption process of insect-based feed, two emerged as the most relevant: the initial investment [7] and regulatory constraints that restrict upscaling and effective circularity [8].

This study examines the adoption process of *Hermetia illucens* meal in Italian trout farms using the Awareness Knowledge Adoption Implementation Effectiveness sequence (AKAIE) sequence, according to the framework of Vecchio et al. [9]. The benefits of implementing the AKAIE sequence depend on the post-adoption steps, which are little addressed by researchers and often not included in the proposed adoption models.

The results delineated the profiles of experimenters, adopters, and non-adopters, shedding light on the “neutrality” of this incremental innovation. By providing policy implications for bolstering the knowledge infrastructure and assisting with paradigm shift strategies, the gathered results can increase awareness of innovation's role in addressing the future challenges of the Italian aquaculture sector.

The present study is part of the outcomes of the Acquainnova 1.0 Project. It serves as the pilot framework for developing Acquainnova 2.0 [10], on which the collected data will be used to build a cost-benefit analysis for different novel feeds.

[1] Manganda, A. S., Sehnem, S., & Lara, A. C. (2024). Transition to the Circular Economy: Innovative and Disruptive Production Technologies Adopted by Agribusiness Startups. *Environmental Quality Management*, 34(1), e22293.

[2] Adamseged, M. E., Ferguson, R., Grundmann, P., & Langendahl, P. A. (2022) Innovative Entrepreneurial Initiatives for Sustainable Food Production and Institutional Change. Available at SSRN 4115179.

[3] Grimsby, S. (2020). New novel food regulation and collaboration for innovation. *British Food Journal*, 123(1), 245-259.

[4] Rogers, E. M., & Murcott, S. (1995). Attributes of innovations and their rate of adoption. *Diffusion of innovations*, 4, 204-251.

[5] Lamberti, L., El Bilali, H., & Belsanti, V. (2011). Contribution of small-scale farmer innovations to sustainable agriculture. *Proceedings–Full Papers*, 118.

[6] Mulazzani, L., Madau, F.A., Pulina, P., Malorgio, G. (2021). Acceptance of insect meal in aquaculture feeding: A stakeholder analysis for the Italian supply chains of trout and seabass. *J World Aquac Soc.* 52: 378–394.

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- [7] Rumbos, C. I., Mente, E., Karapanagiotidis, I. T., Vlontzos, G., & Athanassiou, C. G. (2021). Insect-based feed ingredients for aquaculture: A case study for their acceptance in Greece. *Insects*, 12(7), 586.
- [8] Maulu, S., Langi, S., Hasimuna, O. J., Missinhoun, D., Munganga, B. P., Hampuwo, B. M., Ndakalimwe Naftal G., Mabrouk E., Van Doan H., Kari Z.A., Dawood, M. A. (2022). Recent advances in the utilization of insects as an ingredient in aquafeeds: A review. *Animal Nutrition*, 11, 334-349.
- [9] Vecchio, Y., Masi, M., & Adinolfi, F. (2023). From the AKAP to AKAIE model to assess the uptake of technological innovations in the aquaculture sector. *Reviews in Aquaculture*, 15(2), 772-784.
- [10] <https://acquainnova.org>

Unlocking the Economic Potential of Neglected and Underutilized Fish Species as Food

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Fishing is essential for food security and livelihoods, yet rising demand - driven by population growth, health awareness, and dietary shifts - places increasing pressure on marine ecosystems. Overexploitation is a major concern, with 60% of global fish stocks at their sustainable limit and 30% overexploited [1]. The Mediterranean and Black Sea face even greater challenges, with 60% [2] of stocks in critical condition, while in Italy, this figure reaches 80.4% [3]. Bycatches, including unwanted catches, exacerbate marine biodiversity loss, as many fish are discarded due to quotas, size restrictions, or low market value, leading to waste and inefficient resource use. It has been estimated that 7 to 10 million Tons of commercial fishery catches are discarded yearly worldwide [4].

Neglected and Underutilized Species (NUS) are a significant part of these discards [5,6], often overlooked due to low market demand and value [7]. Despite the EU's landing obligation policy [8], which aims to reduce discards, the issue persists, particularly affecting small-scale fishers who may struggle with additional costs due to the need to adopt new tools and technologies [9]. Within this context, the present study aims to analyze the economic potential of seven considered fish NUS in Italy, in particular: *Boops boops* (Bogue), *Liza ramada* (Thinlip Mullet), *Mullus barbatus* (Red Mullet), *Pagellus erythrinus* (Common Pandora), *Phycis blennoides* (Greater Forkbeard), *Trachurus trachurus* (Atlantic Mackerel), and *Uranoscopus scaber* (Stargazer). A focus group of experts has identified the proposed species as a part of the activities of the project "NUS are good news - Innovative food from Neglected and Underutilized - fish - Species" (Prot. 2022NX5FL8), which aims to convert discarded NUS into a valuable alternative for human consumption such as ready-to-eat "cured seafood products".

The results highlight how, among the seven analyzed species, five have reported a considerable potential loss of value corresponding to €25.7 million, equivalent to 8,765 Tons of discards over the 2020 to 2022 period. These findings underscore how valorizing NUS discards may represent a winning strategy in reducing waste, promoting sustainable fisheries by alleviating pressure on overfished stocks and enhancing biodiversity conservation while creating new market opportunities, in line with the European Green Deal [10]. Moreover, given the limited consumer purchase choices toward a few restricted species categories in EU-27 and Italy, incorporating NUS in the seafood market under an alternative and unusual shape, may also improve consumer acceptance of these less commercial species.

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- [1] SOFIA. (2022). The State of World Fisheries and Aquaculture 2022. FAO. <https://doi.org/10.4060/cc0461en>
- [2] FAO. (2023). Overfishing in the Mediterranean and Black Sea falls to lowest level in a decade. <https://www.fao.org/newsroom/detail/overfishing-in-the-mediterranean-and-black-sea-falls-to-lowest-level-in-a-decade/en>
- [3] ISTAT. (2023). La banca dati dell'Istituto Nazionale di Statistica. <https://www.istat.it/storage/rapporti-tematici/sdgs/2023/goal14.pdf>
- [4] European Commission. (2025). Discarding in fisheries. Oceans and Fisheries. https://oceans-and-fisheries.ec.europa.eu/fisheries/rules/discarding-fisheries_en
- [5] Venugopal, V., Shahidi, F., & Lee, T. (1995). Value-added products from underutilized fish species. *Critical Reviews in Food Science and Nutrition*, 35(5), 431–453. <https://doi.org/10.1080/10408399509527708>
- [6] Kendler, S., Thornes, F. W., Jakobsen, A. N., & Lerfall, J. (2023). Nutritional profiling and contaminant levels of five underutilized fish species in Norway. *Frontiers in Nutrition*, 10. <https://doi.org/10.3389/fnut.2023.1118094>
- [7] Witkin, T., Dissanayake, S. T. M., & McClenachan, L. (2015). Opportunities and barriers for fisheries diversification: Consumer choice in New England. *Fisheries Research*, 168, 56–62. <https://doi.org/10.1016/j.fishres.2015.03.019>
- [8] European Parliament, & European Council. (2013). Regulation (EU) 1380/2013, Article 15, p. 15. <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02013R1380-20230101>
- [9] Vecchio, Y., Masi, M., Del Giudice, T., De Rosa, M., & Adinolfi, F. (2024). Technological innovation in fisheries and aquaculture: What are the “discourses” of the Italian policy network?. *Marine Policy*, 159, 105947–105947. <https://doi.org/10.1016/j.marpol.2023.105947>
- [10] Communication from the Commission to the European Parliament, the European Council, the council, the European economic and social committee and the committee of the regions: The European Green Deal, (2019). https://eur-lex.europa.eu/resource.html?uri=cellar:b828d165-1c22-11ea-8c1f-01aa75ed71a1.0002.02/DOC_1&format=PDF

Assessment of plasma concentrations of procalcitonin (PCT), protein carbonylated content (PCC), symmetric dimethylarginine (SDMA) and asymmetrical dimethylarginine (ADMA) in healthy dairy cows during lactation.

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Procalcitonin (PCT), carbonylated protein content (PCC), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) have been investigated as potential biomarkers for bacterial infections and inflammation in cattle, showing promising results [1-3]. Their concentrations might be influenced by lactation, likely due to physiological inflammation and oxidative stress in dairy cows during this period [4]. This study aimed to evaluate how plasma levels of PCT, PCC, ADMA, and SDMA change in healthy lactating dairy cows at different days in milk (DIM): 15 DIM (T0), 60 DIM (T1), and 150 DIM (T2) (Institutional Animal Care and Use Committee of the University of Pisa, protocol No. 2825/2014).

The study included healthy Italian Friesian cows selected based on physiological dry periods, normal clinical examinations, and specific udder health criteria (California Mastitis Test <1 and somatic cell count <150,000 cells/ml for primiparous and <250,000 cells/ml for multiparous cows) at each time point. Cows that developed mastitis or other illnesses during the study were excluded. Blood samples were collected in lithium heparin tubes at T0, T1, and T2, and plasma was stored at -80 °C. PCT, PCC, ADMA, and SDMA were measured using previously described methods [1-3]. Data distribution was assessed using the Shapiro-Wilk test, and results were reported as medians and interquartile ranges (25%P - 75%P). One-way ANOVA was used to analyze potential physiological variations in biomarker concentrations, with statistical significance set at $p < 0.05$.

A total of 21 cows were included for biomarkers analysis. Median PCT concentrations were 64.29 pg/ml (40.00–143.23), 75.36 pg/ml (40.00–161.47), and 77.5 pg/ml (40.00–120.18) at T0, T1, and T2, respectively. Median PCC concentrations were 0.17 nmol/ml/mg (0.10–0.27), 0.14 nmol/ml/mg (0.08–0.23), and 0.20 nmol/ml/mg (0.08–0.22) at T0, T1, and T2, respectively. Median ADMA concentrations were 0.11 $\mu\text{mol/l}$ (0.09–0.15), 0.11 $\mu\text{mol/l}$ (0.09–0.13), and 0.10 $\mu\text{mol/l}$ (0.09–0.14) at T0, T1, and T2, respectively. Median SDMA concentrations were 0.11 $\mu\text{mol/l}$ (0.09–0.14), 0.12 $\mu\text{mol/l}$ (0.09–0.15), and 0.10 $\mu\text{mol/l}$ (0.09–0.16) at T0, T1, and T2, respectively. No statistically significant differences were observed in any biomarker across the three time points.

Our results suggest that, despite variations in oxidative stress observed during different lactation stages in other studies, these changes might not affect the biomarkers. Future studies are needed to establish reference ranges, but lactation stage may not be required. Distinct reference ranges for plasma PCT, PCC, ADMA, and SDMA across lactation stages may not be necessary.

[1] Bonelli et al. Case-Control study: Evaluation of plasma procalcitonin concentration as an indicator of inflammation in healthy and sick cows. *Research in veterinary science*, 155, 56-61, 2023.

[2] Sala et al. Case-control study: endogenous procalcitonin and protein carbonylated content as a potential biomarker of subclinical mastitis in dairy cows. *Veterinary Sciences*, 10(12), 670, 2023.

[3] Teerlink, T. HPLC analysis of ADMA and other methylated L-arginine analogs in biological fluids. *Journal of Chromatography B*, 851(1-2), 21-29, 2007.

[4] Tufarelli, V. et al. Biological health markers associated with oxidative stress in dairy cows during lactation period. *Metabolites*, 13(3), 405, 2023.

Impact of Milking practices on the Welfare of Dairy Camels: A Behavioural and Physiological approach

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Traditionally, hand milking has been the standard practice in camel farming. However, the introduction of machine milking has emerged as a key solution to meet the growing demand for camel milk. Despite its advantages, machine milking causes various stressors [1] that can impair the animals' behavioural responses. This study aims to assess the effect of milking practice manual vs. mechanical on the behavioural responses of camels. Twelve intensively reared Maghrebi dairy camels (aged 4 to 19 years, Body weight = 439.1 ± 35.7 kg at the start of lactation) at IRA-Chenchou experimental station were observed. Their daily diet included 5 kg of oat hay, 3 kg of alfalfa hay, 8 kg of wilted green alfalfa, and 2 kg of concentrate, with free access to water. Before transitioning to machine milking, camels were milked by hand for two weeks twice daily in the presence of their calves to stimulate milk ejection. Machine milking was thereafter introduced. Milking was performed twice a day in 2x3 herringbone parlour specially designed for camels. Milk ejection was either stimulated by the milking liner or by an intravenous injection of oxytocin (10 IU) only when needed. Milking followed a standardized routine, with machine settings adjusted according to Atigui et al. [2]. Continuous direct observations during milking sessions were noted and behavioural data were collected to assess the camels' responses. Data analysis was conducted using SAS software (v 9.0) via the GLM procedure with a multi-factor analysis of variance. Results indicated that stress-related behaviours were more prevalent during machine milking (figure1). Kicking and stepping were significantly affected by milking practices ($P < 0.001$). Conversely, no significant difference in vocalization behaviour was observed between manual and mechanical milking (5.04 ± 0.80 vs. 5.64 ± 0.60 , respectively). Similarly, no difference was recorded ($P = 0.60$) for the vigilance status of the camels. Conversely, the stress state caused a blockage of the natural release of oxytocin resulting in a significantly higher need for exogenous oxytocin (figure2) to ensure milk ejection and total emptying of the camel's udder.

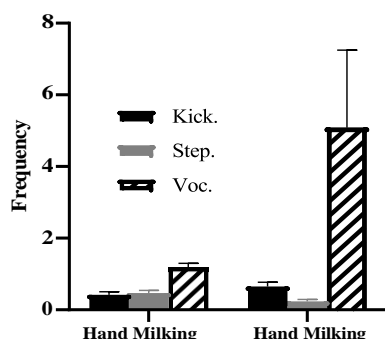


Figure 1: Effect of milking practice on some camels' behavioural response (Kick = Kicking, Step = stepping, Voc. = Vocalization).

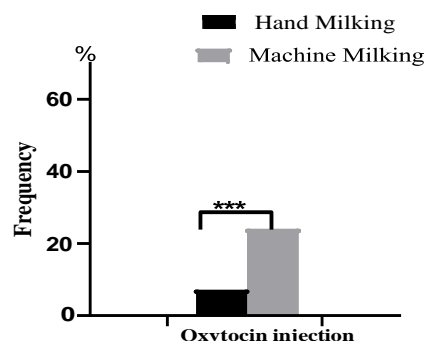


Figure 2: Effect of milking practice on state of vigilance and need for OT injection ($\chi^2 = 3.04$; $P = 0.055$ and $\chi^2 = 25.80$; $P < 0.0001$ respectively)

Conversely, behaviours associated with well-being were more frequent during hand milking, with camels ruminating more often with longer rumination cycles (3.29 cycles and 112.73 s, respectively) compared to machine milking (2.22 cycles and 67.5 s, respectively). In conclusion, these findings suggest that although the response to mechanical milking induces stress, a training period preceded by a phase of manual milking can improve the behavioural and physiological responses of dairy camels.

[1] F. Saltalamacchia, C. Tripaldi, A. Castellano, F. Napolitano, M. Musto, G. De Rosa, Animal Welfare, 2007, DOI: <https://doi.org/10.1017/S0962728600031183>.

[2] M. Atigui, P.G Marnet., A. Barmat, T. Khorchani, M. Hammadi, Tropical Animal Health and Production, 2015, DOI: 10.1007/s11250-014-0708-0.

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Optimization of Holstein Cow Lactation Curves in Tunisia: Impact on Milk Production and Animal Welfare

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Dairy farming plays a crucial role in Tunisia's agricultural sector. Understanding lactation curves is essential for optimizing milk yield while ensuring animal welfare, as they offer insights into cows' physiological responses, aiding in better management of feeding, reproduction, and health [1, 2]. This study aimed to adjust the lactation curves of Holstein cows using the Wood model and analyze the factors influencing these curves. It also assessed how environmental and management factors affect milk yield, fat, and protein content, while highlights the role of optimized lactation management can reducing stress and improving cow longevity. The study utilized data from 190,905 daily milk yield records collected across ten herds from four different production sectors. The dataset included parameters such as milk yield, fat percentage, protein percentage, calving date, and lactation number. Nonlinear regression analysis using the Gauss-Newton algorithm was performed. and a mixed linear model was used to assess the impact of sector, herd, calving season, and lactation number on lactation parameters. The results showed that milk yield, fat, and protein content varied significantly across lactation numbers and environmental conditions. Peak production was observed between the third and fifth lactation, with an average daily milk yield of 30.4 kg at peak during the third lactation. In contrast, the highest lactation persistence was recorded during the first lactation, indicating a slower decline in milk production post-peak. Environmental factors played milk production, with cows calving in winter and spring exhibited better initial yield and persistence, likely due to favorable conditions [3]. Conversely, cows calving in summer showed lower yields, possibly due to heat stress and lower-quality forage. Herd management practices, including feeding and milking techniques, also played a significant role [2]. From an animal welfare perspective, prolonged high milk production can cause metabolic stress and increased susceptibility to diseases such as mastitis. Proper lactation curve management, including balanced nutrition and optimal dry periods, is essential to maintaining cow health [4]. This study demonstrates the importance of adjusting lactation curves to improve milk yield while considering factors that influence animal welfare. The Wood model effectively captures variations in milk production across lactations and management conditions. The findings highlight the need for strategic herd management, including seasonally adjusted feeding programs and controlled breeding cycles, to optimize milk yield while ensuring cow well-being. Future research should explore precision dairy technologies to further enhance lactation management and minimize stress-related health issues, ensuring sustainable dairy farming in Tunisia.

[1] P.D.P. Wood, Algebraic model of the lactation curve in cattle. *Nature*, 216, pp. 164-165, 1967.

[2] B. Rekik, A. Ben Gara, M. Ben Hamouda, H. Hammami, Fitting lactation curves of dairy cattle in different types of herds in Tunisia. *Livestock Production Science*, 83, pp. 309-315, 2003.

[3] G. Agabriel, J.B. Coulon, G. Marty, N. Cheneau, Factors influencing milk protein content in dairy cows. *INRA Production Animale*, 3, 137-150, 1990.

[4] M.T. Sraïri, M. Ben Salem, A. Bourbouze, M. Elloumi, B. Faye, Sustainability perspectives of dairy cattle farming in North Africa. *Colloque International Développement Durable des Productions Animales*, 20-21, 2008.

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Monitoring metabolic and hormonal changes in Sarda rams throughout the breeding season: A key to optimizing their management and productivity

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Figure 1- Grazing rams

During the breeding season of Sarda rams (June to December), they must cope with changes in housing conditions, feeding regimens, social environment, and ambient temperatures. This study aimed to evaluate quantitative markers of energy balance and social, environmental or nutritional stress that can offer important insights into rams' physiological responses to these conditions. Twenty-one Sarda rams (2-6 years of age) were examined at ~40-day intervals from June to December. All rams received three s.c. slow-release melatonin implants between early April and late May to advance a mating period and increase their total fertility rate. Circulating concentrations of non-esterified fatty acids (NEFA), urea, triglycerides, cholesterol and testosterone as well as fecal thyroid hormone (FTM) and cortisol (FCM) metabolites were measured. Body condition score (BCS) was assessed by measuring muscle development and subcutaneous fat on a scale from 1 (emaciated) to 5 (obese). Blood metabolites were measured with commercial kits and the BS-200 Mindray clinical chemistry analyser, while FTM and FCM were assayed using a commercial ELISA kit and EIA, respectively. Circulating concentrations of testosterone were determined by radioimmunoassay. Mean BCS was higher ($P<0.001$) in July (3.10 ± 0.06) than in September (2.87 ± 0.06) and November (2.88 ± 0.06). Cholesterol and NEFA concentrations peaked ($P<0.001$) in November (0.42 ± 0.06 mmol/L and 51.6 ± 1.9 mg/dL, respectively) but were lower ($P<0.05$) in September (0.14 ± 0.06 mmol/L and 42.0 ± 1.9 mg/dL, respectively) and December (0.13 ± 0.06 mmol/L and 42.8 ± 1.9 mg/dL, respectively). Triglyceride levels were lower ($P<0.05$) in November (17.7 ± 2.02 mg/dL) than in July (25.7 ± 2.02 mg/dL) and December (26.8 ± 2.02 mg/dL). Mean urea levels were higher ($P<0.0001$) in June (23.9 ± 1.3 mg/dL) and July (23.7 ± 1.3 mg/dL) than in all other months (17.1 ± 1.3 , 17.9 ± 1.3 , 14.5 ± 1.3 mg/dL for September, November, and December, respectively). Fecal thyroid hormone metabolite levels were higher ($P<0.001$) in June (82.5 ± 4.7 ng/g feces) compared to July (61.9 ± 4.7 ng/g feces) and December (55.8 ± 4.7 ng/g of feces), and FCM levels were higher ($P<0.05$) in November (424 ± 42.6 ng/g of feces) compared to September (259 ± 43.6 ng/g of feces). Blood testosterone concentrations were higher ($P<0.05$) in June (6.02 ± 1.1 ng/mL) than in July, September, and November (2.09 ± 0.4 , 1.85 ± 0.3 , and 2.32 ± 0.4 ng/mL, respectively). In summary, various metabolic and hormonal parameters fluctuate significantly during the breeding season of Sarda rams, coinciding with specific changes in their management (e.g., elevated levels of NEFA, cholesterol, and FCM at the time of removal from the flock in November). This knowledge can lead to more effective management of Sarda rams, especially during the periods of high metabolic energy requirements.

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The importance of Bovine tuberculosis genetic resistance in improving animal welfare

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a major significant health concern affecting both livestock and humans. The disease poses significant economic challenges and animal welfare issues, leading to reduced productivity, mandatory culling, and potential suffering in infected animals. Selective breeding for genetic resistance could be a sustainable approach to disease control. This study investigates the association between polymorphisms in the SLC11A1 and CARD15 genes and susceptibility to bTB in Holstein cattle in Tunisia. These genes play a role in immune responses, potentially influencing resistance or vulnerability to infection. A total of 200 Holstein cows were studied, including 50 tuberculosis-positive cases and 150 healthy controls. The research was conducted following strict animal welfare guidelines, ensuring ethical handling and minimizing stress for the animals. Diagnosis of tuberculosis was carried out using a tuberculin skin test to identify infected individuals, with clinical symptoms such as weight loss and respiratory distress were considered to ensure accurate classification. For genetic Analysis, Blood samples were collected and processed for PCR-RFLP analysis to identify polymorphisms in the SLC11A1 and CARD15 genes. Statistical tests were then used to assess associations between these genetic variants and susceptibility to the disease. The SLC11A1 Gene analysis revealed that the GG genotype was associated with higher resistance to bTB, whereas the CC and CG genotypes were linked to increased susceptibility. This suggests that the G allele may play a protective role by enhancing immune responses. Regarding the CARD15 Gene, the AA and AG genotypes were more frequently observed in infected cattle, indicating higher susceptibility, while the GG genotype was associated with resistance to the disease. These findings support the idea that genetic selection could improve disease resistance, reducing the need for culling and minimizing animal suffering. Prior studies, such as those by Wang et al. [1] and Allen et al. [2], have similarly identified these genes as important factors in tuberculosis resistance. The study confirms a strong association between polymorphisms in these genes and bTB susceptibility in Holstein cattle. This knowledge can be used to improve selective breeding programs, helping to reduce the prevalence of bTB and improving animal welfare by limiting disease-related suffering. Future research should focus on gene expression studies and expand to larger cattle populations to further refine selection strategies for disease-resistant livestock.

[1] Wang, Y. Wang, S. Liu, T. Tu, W. Li, W. Dong, G. Xu, C. Qin, B. Liu, K. Yang, J. Chai, X. Shi, Y. Zhang, CARD15 Gene Polymorphisms Are Associated with Tuberculosis Susceptibility in Chinese Holstein Cows, PLOS ONE, 10, 2015.

[2] A.R. Allen, G. Minozzi, E.J. Glass, R. A. Skuce, S.W.J. McDowell, J.A. Woolliams, S.C. Bishop, Bovine tuberculosis: The genetic basis of host susceptibility, P. Roy. Soc. B, 277, 2737–2745, 2010.

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Livestock animal hair as indicator of environmental heavy metals pollution in Central Albania

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Large areas of Albania are developed on serpentine rocks, resulting in significant reserves of iron, nickel, chromium, and copper in the soil. Combined with the presence of mines and metal foundries [1], this represents a risk of heavy metals exposure for both animals and humans, endangering their health and welfare. Moreover, as Albania works to align with *EU acquis* on environmental management, food safety, and veterinary policy, further research in these areas is crucial.

Animal hair is an excellent bioindicator of environmental heavy metal pollution, and its sampling provides a simple, non-invasive method for assessing animal health and welfare [2]. Therefore, this study aims to evaluate and compare the level of the main heavy metals (arsenic, cadmium, chromium, copper, iron, nickel, lead, and zinc) in the hair of bovines farmed in the two Counties that Central Albania comprises (Tirana and Elbasan). A total of 25 clinically healthy adult cows were selected from each county, all grazing on local pastures or consuming locally produced fodder. Hair samples were collected from each animal's left abdominal region, just behind the costal arch, and stored in plastic bags at room temperature, protected from light until analysis. Samples were then analyzed by inductively coupled plasma-optical emission spectrophotometer (ICP-OES; PerkinElmer-Optima 7000DV).

No significant differences in element concentrations were found between the Counties. However, as expected given the region's geology, iron, nickel, chromium, and copper levels were particularly high in the cows' hair. Copper levels (17.84 ± 7.75 and 15.84 ± 4.48 mg/kg, in Elbasan and Tirana County, respectively) were significantly higher than those reported in literature, even exceeding those in cows grazing near closed lead-cum-operational zinc smelter [3]. However, the animals showed no signs of copper intoxication, suggesting that the levels were not high enough to cause oxidative damage. On the other hand, lead and zinc had values consistent with the physiological ones [3]. Finally, arsenic concentration (2.08 ± 1.41 and 1.51 ± 0.76 mg/kg, in Elbasan and Tirana County, respectively) was very high compared with what has been reported in previous studies [3-5]. This result was unexpected, considering that the mean concentrations of arsenic in the agricultural and forest soils of Albania are lower than the global mean concentrations [6]. In conclusion, this study highlights potential risks to both human and animal health and welfare associated with heavy metals in the soil of Central Albania. Further research is needed to better assess the actual risks posed by these elements.

[1] S. Shallari, C. Schwartz, A. Hasko, et al., "Heavy metals in soils and plants of serpentine and industrial sites of Albania", *Science of the total environment*, vol. 209, pp.133-142, 1998.

[2] MN. Rashed, ME. Soltan. "Animal hair as biological indicator for heavy metal pollution in urban and rural areas", *Environ Monit Assess*, vol. 110, pp. 41-53, 2005.

[3] RC. Patra, D. Swarup, MC. Sharma, et al., "Trace Mineral Profile in Blood and Hair from Cattle Environmentally Exposed to Lead and Cadmium Around Different Industrial Units", *J. Vet. Med.*, vol. 53, pp. 511-517, 2006.

[4] BK. Datta, MK. Bhar, PH. Patra, et al., "Effect of environmental exposure of arsenic on cattle and poultry in Nadia district, west Bengal, India", *Toxicol Int.*, vol. 19, pp. 59-62, 2012.

[5] A. Das, M. Joardar, NR. Chowdhury, et al., "Arsenic toxicity in livestock growing in arsenic endemic and control sites of West Bengal: risk for human and environment", *Environ. Geochem. Health.*, vol. 43, pp. 3005-3025, 2021.

[6] F. Gjoka, RA. Duerling, and J. Siemens, "Background concentrations and spatial distribution of heavy metals in Albania's soils", *Environ. Monit. Assess.*, vol. 194, pp. 115, 2022.

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How to measure neurons: a pipeline to characterize their shape and morphology

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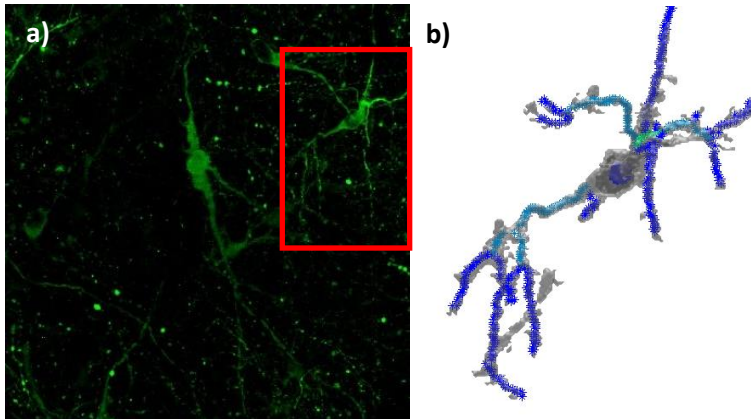


Figure 1: Example of result obtained through the pipeline. a) Confocal acquisition of Tyrosine Hydroxylase-positive Neurons. b) In depth zoom of the cell highlighted in red. In grey is shown the segmentation. Marked with different shades of blue, the Strahler Order (blue for first and light blue for second order) of the final corrected structure of the reconstruction.

Digitalising a high-fidelity map of the neurons in a mammalian brain is a key goal in neuroscience research [1]. Single-neuron reconstructions from empirical data are crucial for studying the normal development of dendritic and axonal arbours, as well as for documenting neuro-pathophysiological changes [2]. Moreover, quantifying the size, shape, and complexity of neurons is essential for assessing the degree of similarity between advanced cellular models, such as brain organoids, and the brain itself.

Undoubtedly, advances in imaging (e.g., confocal microscopy) and imaging protocols (e.g., clarification) have enabled the inspection of large populations of neurons (up to 500 μm) at cellular (sub-micrometric) resolution [3]. Despite these advancements, a major challenge in deciphering the brain's complex

micro-architecture lies in the limited number of segmentation algorithms able to deal with the dense-packed scenarios typical of the mammalian brains, and of organoids. Moreover, such algorithms are difficult to be handled by non-expert users, making the extraction of morphological parameters time-consuming and prone to human errors.

In this light, we developed a pipeline for acquiring, segmenting and characterising neuron morphology. Briefly, samples are immunolabelled to highlight the target structure using antibodies [3]. The following step involves the acquisition of the sample through a confocal microscope, which high resolution allows to capture cell structures in their entirety. Then images are then segmented using SENPAI [4] an algorithm purposely developed to accurately isolate neurons. It exploits pixels intensity and second derivatives to distinguish between background and foreground. The obtained mask is processed with the Watershed algorithm, to split any touching cells. Algorithms are all implemented into an easy-to-use Graphical User Interface developed in Matlab. The resulting reconstructions are further refined with graph analysis to erase any remaining wrong branches. Once cells are reconstructed in their native arrangement within the neuronal tissue, they are characterized by means of morphological parameters (Sholl analysis, Strahler analysis, shape detection) describing their shape, size, and complexity. The GUI functionality was tested on brain organoids, immunolabeled to reveal dopaminergic neurons (i.e. Tyrosine Hydroxylase-positive neurons), and acquired via confocal microscopy. An example of segmentation obtained is shown in Figure 1.

Future developments will include exploiting graph analysis to improve neuron reconstruction, the implementation of a structural network analysis of the tissue and the possibility to isolate population of neurons by means of their morphologic features.

- [1] A. Motta *et al.*, «Dense connectomic reconstruction in layer 4 of the somatosensory cortex», *Science*, vol. 366, fasc. 6469, p. eaay3134, nov. 2019, doi: 10.1126/science.aay3134.
- [2] T. Jürgens *et al.*, «Reconstruction of single cortical projection neurons reveals primary spine loss in multiple sclerosis», *Brain J. Neurol.*, vol. 139, fasc. Pt 1, pp. 39–46, gen. 2016, doi: 10.1093/brain/awv353.
- [3] R. Tomer, L. Ye, B. Hsueh, e K. Deisseroth, «Advanced CLARITY for rapid and high-resolution imaging of intact tissues», *Nat. Protoc.*, vol. 9, fasc. 7, pp. 1682–1697, lug. 2014, doi: 10.1038/nprot.2014.123.
- [4] S. Cauzzo *et al.*, «A modular framework for multi-scale tissue imaging and neuronal segmentation», *Nat. Commun.*, vol. 15, fasc. 1, p. 4102, mag. 2024, doi: 10.1038/s41467-024-48146-y.

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Automatic segmentation of peripheral nerve histological sections for hybrid models of neuromodulation

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Electrical stimulation of peripheral nerves is a promising approach to restore movements and sensations, and modulate the activity of internal organs affected by different pathologies, such as cardiac diseases or lower urinary tract dysfunction [1,2]. Clinical neuromodulation devices make use of large extraneural prostheses [3,4] placed beside or around the nerve, and employing preset electrical stimulation parameters. Intraneural prostheses instead offer higher functional selectivity by targeting individual fascicles within the nerve, enabling precise modulation of specific pathways while minimizing off-target effects [5]. Computational models can be used for optimizing neuromodulation strategies and designing selective neural prostheses, thus a precise understanding of nerve organization is essential [6]. Traditional methods to achieve nerve topography, such as manual segmentation of histological nerve sections, are time-intensive and prone to variability.

In this study, we present advanced computational tools and data-driven approaches to enhance the analysis of peripheral nerve histological sections and their integration into computational models. We developed a U-Net-based deep learning architecture for the automatic segmentation of fascicles from 158 histological sections of swine vagus, pudendal, and sciatic nerves, stained with Toluidine Blue, Hematoxylin and Eosin, and osmium tetroxide. The U-Net architecture leveraged a pre-trained encoder, fine-tuned with a limited dataset, and augmented with rotation, zoom, and flipping to enhance robustness. Segmentation outputs were validated against manual annotations using metrics such as fascicle count and area. The segmented data were integrated into computational models to simulate nerve stimulation and evaluate fascicle-specific recruitment thresholds (current levels needed to activate a percentage of fibers) and selectivity indices (the ability to target specific fascicles).

Our results demonstrated that automatic segmentation closely matched manual annotations, with domain-specific metrics, such as fascicle count and area, remaining within a $\pm 10\%$ error range. Computational models based on automated segmentations accurately reproduced recruitment thresholds and selectivity indices, with deviations of less than 10% in most cases. These findings confirm that segmentation errors minimally impact predictions of stimulation outcomes, ensuring the reliability of the workflow for both fascicle-level and nerve-level analyses.

This work highlights the feasibility of using automated segmentation and modeling to inform electrode design, optimise stimulation protocols, and advance closed-loop neuroprostheses. Such an approach not only accelerates neuroprosthetic development but also contributes to a better understanding of peripheral nerve functional topography, paving the way for personalized neuromodulation therapies.

- [1] R. Assmann *et al.*, "Stimulation Parameters for Sacral Neuromodulation on Lower Urinary Tract and Bowel Dysfunction-Related Clinical Outcome: A Systematic Review," *Neuromodulation: Technology at the Neural Interface*, vol. 23, no. 8, pp. 1082–1093, Dec. 2020, doi: 10.1111/ner.13255.
- [2] V. Dusi, C. Zhu, and O. A. Ajijola, "Neuromodulation Approaches for Cardiac Arrhythmias: Recent Advances," *Curr Cardiol Rep*, vol. 21, no. 5, p. 32, May 2019, doi: 10.1007/s11886-019-1120-1.
- [3] N. Kohli and D. Patterson, "InterStim® Therapy: A Contemporary Approach to Overactive Bladder," p. 10.
- [4] A. Mertens, R. Raedt, S. Gadeyne, E. Carrette, P. Boon, and K. Vonck, "Recent advances in devices for vagus nerve stimulation," *Expert Review of Medical Devices*, vol. 15, no. 8, pp. 527–539, Aug. 2018, doi: 10.1080/17434440.2018.1507732.
- [5] M. Badi and S. Wurth, "Intrafascicular peripheral nerve stimulation produces fine functional hand movements in primates," *SCIENCE TRANSLATIONAL MEDICINE*, vol. 13, p. 16, 2021, doi: 10.1126/scitranslmed.abg6463.
- [6] S. Romeni, G. Valle, A. Mazzoni, and S. Micera, "Tutorial: a computational framework for the design and optimization of peripheral neural interfaces," *Nat Protoc*, vol. 15, no. 10, pp. 3129–3153, Oct. 2020, doi: 10.1038/s41596-020-0377-6.

Hybridizing photogrammetry techniques and AI to produce a 3D printing realistic replica of the horse colon.

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The three-dimensional (3D) visualization of anatomical structures and the study of their topographical relationships represent a significant challenge in veterinary medical education, particularly for complex regions such as the equine caecum and colon. To date, no existing technology provides a 3D-printable model of this anatomically and pedagogically essential region. This project seeks to address this gap by implementing a hybrid methodology that combines photogrammetry with artificial intelligence (AI) to develop a highly realistic and accurate replica of the equine colon. This initiative adheres to the principles of the 3Rs (Replacement, Reduction, Refinement) and upholds biosafety standards. By delivering an accessible, realistic, and 3D-printable anatomical model, this approach reduces the reliance on animal dissections for educational purposes, thereby limiting the use of animal specimens. Research in educational pedagogy has demonstrated that the integration of physical 3D models significantly enhances the acquisition of anatomical knowledge. The manipulation of three-dimensional representations fosters a deeper understanding of the complex spatial relationships among anatomical structures and promotes cognitive engagement in learners ^[1]. The methodology begins with the creation of a plasticine model, sculpted to replicate the anatomical features of the caecum and colon with high precision.



Figure 1. Photography of 3D printing first version (without taeniae)

Photographs of this model were captured from five distinct angles and processed using software embedded with AI algorithms (Hyper3D Rodin 1.4) to generate an accurate 3D digital representation optimized for 3D printing. Key anatomical landmarks, including taeniae and haustra, were highlighted using (Mesh Inspector 2.4.8). A PRUSA XL printer produced a hollow prototype measuring 15 × 8 × 8 cm, with a weight of 300 g, at a cost of €5. A full-scale model, weighing approximately 5 kg, is estimated to cost less than €100. Additionally, the model includes strategically designed windows to provide internal visualization of critical structures such as the ileocaecal papilla and the caecocolic orifice.

This work proposes the development of a life-size 3D-printed model of the caecum and ascending colon to be integrated within an equine skeletal framework, enabling veterinary students to gain a precise understanding of topographical anatomy. Furthermore, a reduced-scale version of the model, designed as an open-access resource and easily manipulable, is intended to support self-directed learning outside of the classroom setting.

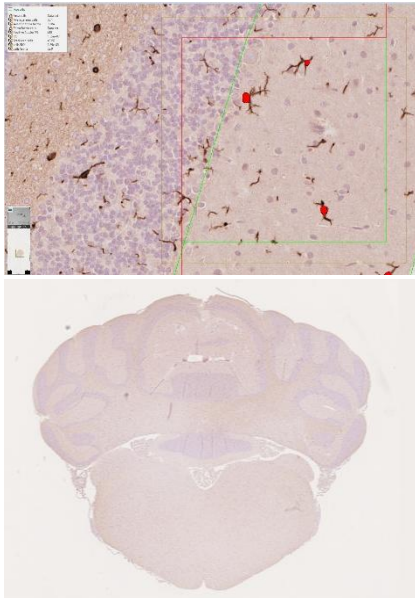
[1] A. Yousef, D. Manisha, S. Stéphane, and S. Goran, "The application of 3D printing in anatomy education," *Med Educ Online*, vol. 20 (1), 2015, [Online]. Available: <https://doi.org/10.3402/meo.v20.29847>

Morphometric Analysis of Cerebellar Microglia in Male and Female Rats Using AI-Based Deep Learning

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Microglia, the resident immune cells of the central nervous system (CNS), play a crucial role in maintaining brain homeostasis. It becomes activated in response to injury or infection, undergoing significant morphological changes. Since these structural transformations reflect their functional state and activation dynamics, analysing microglial morphology is essential [1]. We hypothesize that research on microglial cells in the male and female brain may contribute to a deeper understanding of their sex- and region-specific roles in modulating neuronal activity and neuroimmune responses in both healthy and pathological conditions. These types of investigations are particularly important because the sex variable has often been overlooked or disregarded in biomedical research, leading to the underrepresentation of females in animal models of disease and hindering our understanding of female biology [2].

This study examined sex-linked differences in cerebellar microglia in male and female rats through a detailed morphometric analysis. Microglial cells were identified using the anti-Iba1 antibody as an immunohistochemical marker. We analyzed key morphological parameters in Iba1-immunostained cells, including cell density (cells/mm²), soma area (μm²), and shape factor, which offer insights into microglial complexity and structure.

A total of 10 adult rats (5 females, 5 males) were analyzed, with batch processing performed on 200 sections, leading to the identification of 179.775 microglial cells. To ensure a comprehensive assessment, measurements were taken from three distinct regions of the cerebellar cortex: white matter (WM), granular layer (GL), and molecular layer (ML), allowing a region-specific evaluation of microglial morphology.

High-resolution whole-slide images were acquired using a NanoZoomer Hamamatsu slide scanner at 20× magnification with automatic focusing. Image processing was performed with the AI-powered Visiopharm software (Hoersholm, Denmark, v2023.01) to enable precise morphometric analysis. A deep learning-based training process was employed for accurate cerebellar layer recognition, automated quantification of Iba1-positive microglia, and assessment of morphological parameters. Multiple training iterations ensured reliable differentiation of microglia from other cell types, enhancing analytical accuracy and consistency.

By leveraging this artificial intelligence-driven approach, we generated a robust and comprehensive dataset, enhancing the reliability of our findings on sex-linked differences in microglial morphology. Our results revealed higher morphological parameter values in females compared to males; however, within the three cerebellar areas, the parameters displayed a similar pattern in both males and females. These findings offer insights into sex-related variations in microglial structure and function, providing a foundation for more targeted research into CNS diseases and the development of pharmacological treatments that consider sex-specific differences.

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[1] D.P. Schafer, E.K. Lehrman, A.G. Kautzman, R. Koyama, A.R. Mardinly, R. Yamasaki, R.M. Ransohoff, M.E. Greenberg, B.A. Barres, and B. Stevens, "Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner," *Neuron*, Vol. 74(4), pp. 691-705, 2012.

[2] R.M. Shansky, and C.S. Woolley, "Considering Sex as a Biological Variable Will Be Valuable for Neuroscience Research," *Jour. Neurosci*, vol. 36(47), pp. 11817-11822, 2016.

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Deep learning based approach to quantify cerebellar neuronal cell loss in feline neurological diseases: a new potential tool to overcome subjective evaluation

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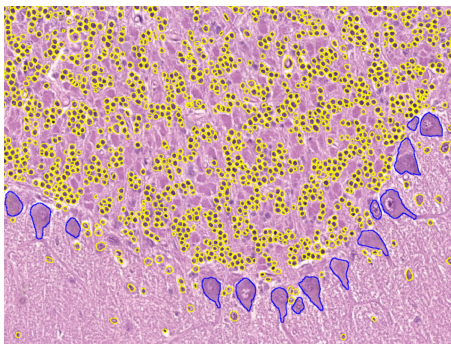


Figure 1: Example of cell segmentation on a 1024x1024 region of interest.

One of the most promising applications of artificial intelligence (AI) is addressing issues related to subjective evaluations in veterinary pathology. This is particularly relevant in neuropathology, where critical elements of histopathological assessment, such as the quantification of neuronal loss or the degree of glial proliferation (astrogliosis), usually rely on the subjective judgment of the operator.

To tackle this issue, we focused our efforts on the quantification of neuronal loss in each layer of the cerebellar cortex, associated with various degenerative, inflammatory, malformative and space-occupying cerebellar lesions in cats.

Here, we report the preliminary results of an AI-based approach to objectively quantify cerebellar neuronal loss in two feline cases: a lysosomal storage disease (LSD)[1] and a cerebellar abiotrophy (CA)[2]. Although the two neurological conditions show cerebellar atrophy, a precise quantification of cerebellar neuronal cell loss has never been performed.

Both cats were referred for progressive neurological symptoms. The LSD case showed marked ataxia and mild occasional tremors while histology revealed widespread intraneuronal vacuolations in the brain and spinal cord, along with cerebellar atrophy due to neuronal loss. Histological analysis of the CA case revealed diffuse atrophy of the cerebellar cortex involving all three layers.

Cells in each histological sample were identified using a convolutional neural network, specifically the CISCA segmentation framework[3]. CISCA was trained on Nissl-stained annotated patches from the CytoDark0 dataset[4]. To enhance its performance on H&E-stained Whole Slide Images (WSIs), we applied stain augmentation to the training set and refined CISCA's segmentations by annotating additional H&E-stained patches. These H&E-stained patches were carefully annotated to delineate cell contours and distinguish Purkinje cells from other cell types. Once trained, the CISCA model was applied to our samples. WSIs were downsampled and normalized to match the staining appearance of an H&E reference image. The CISCA framework was then used to segment and identify cells in the normalized samples. Cerebellar layers were manually annotated using QuPath, and cells were assigned to their respective layers through morphological reconstruction. Finally, cell density for each layer was computed as the ratio of total detected cells to the layer's area. So far, the results reveal a significant reduction in neuronal density in the cases with cerebellar pathology compared to the controls, in both the Purkinje and granular cell layers.

This study marks an initial step toward developing a reliable, quantitative AI-driven model, designed to be universally applicable across different neurological diseases and adaptable to multiple species.

- [1] M. Castagnaro, J. Alroy, A. A. Ucci, and R. H. Glew, "Lectin histochemistry and ultrastructure of feline kidneys from six different storage diseases," *Virchows Arch B*, vol. 54, pp. 16–26, 1987, doi: 10.1007/BF02899193.
- [2] A. Negrin, M. Bernardini, W. Baumgärtner, and M. Castagnaro, "Late onset cerebellar degeneration in a middle-aged cat," *Journal of feline Medicine and Surgery*, vol. 8 (6), pp. 424–9, 2006, doi: 10.1016/j.jfms.2006.04.007.
- [3] V. Vadori, J.-M. Graïc, A. Peruffo, G. Vadori, L. Finos, and E. Grisan, "Cisca and cytodark0: a cell instance segmentation and classification method for histo(patho)logical image analyses and a new, open, nissl-stained dataset for brain cytoarchitecture studies," *arXiv preprint arXiv:2409.04175*, 2024.
- [4] V. Vadori, J.-M. Graïc, A. Peruffo, G. Vadori, L. Finos, and E. Grisan, "Cytodark0," *Zenodo*, (<https://doi.org/10.5281/zenodo.13694738>), 2024.

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Deep Learning-Based Cell Segmentation on Nissl-Stained Histology Slices to Quantify Sexual Dimorphisms in the Cytoarchitecture of the Chimpanzee Cerebellum

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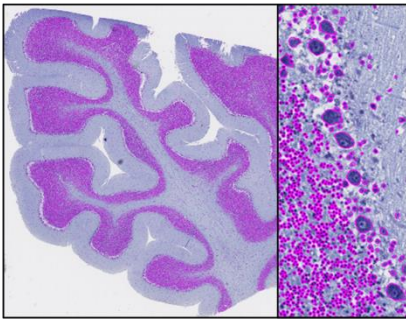


Figure 1. An example of a WSI from the Chimpanzee Cerebellum with cells segmented using the CISCA model.

Sexual dimorphism in primates is observed in various morphological traits, such as body size, craniofacial structure, and skeletal dimensions, as well as within the brain [1]. However, the extent of sexual dimorphism within the cerebellar cytoarchitecture of chimpanzees remains underexplored. This study aims to quantify morphological and topological differences in cerebellar cells between male and female chimpanzees (*Pan troglodytes*) using deep learning (DL) techniques.

To achieve this, we employed CISCA [2], a DL framework designed for automatic cell instance segmentation and classification in histological images. In CISCA, a whole slide image (WSI) of any size is first divided into overlapping 256×256 patches with 50% overlap. Each patch is processed by CISCA-Net, a U-Net-based DL model with three decoder heads. The first head classifies pixels into cell bodies, boundaries, and background; the second regresses four directional distance maps; and the third classifies individual cells into types. The outputs from the decoder are combined

through a tailored post-processing pipeline to achieve accurate cell segmentation across the entire WSI.

CISCA-Net was trained on CytoDARK0 [3], the first publicly available, annotated dataset of Nissl-stained histological images of the mammalian brain. This dataset includes samples from the cortex, cerebellum, and hippocampus of species within the Cetartiodactyla and Primates orders, featuring a total of 38,755 annotated cells. CytoDARK0 serves as a robust foundation for training DL models aimed at studying brain cytoarchitecture.

By applying CISCA to Nissl-stained WSIs of the chimpanzee cerebellum, we achieved precise segmentation of individual cells, enabling comprehensive morphometric analyses with minimal human bias. From each segmented cell, we extracted quantitative attributes such as area, axis ratio, and local cell density to compare different cerebellar regions [4]. Preliminary findings reveal subtle yet statistically significant dimorphic patterns in cell morphology and spatial distribution within the cerebellar cortex, suggesting potential sex-based differences in cerebellar structure and function.

This study highlights the effectiveness of DL models like CISCA, trained on specialized datasets (e.g., CytoDARK0), in facilitating quantitative analyses of brain cytoarchitecture. Automated cell segmentation enhances morphological study efficiency and provides a reproducible framework for identifying structural variations related to function and behaviour. More broadly, advanced image analysis techniques expand insights beyond traditional histological cell counting, offering a powerful tool for investigating primate brain structure and its inherent variability.

[1] J. M. Abel, D. M. Witt, and E. F. Rissman, "Sex differences in the cerebellum and frontal cortex: Roles of estrogen receptor alpha and sex chromosome genes," *Neuroendocrinology*, vol. 93, no. 4, pp. 230–240, 2011, doi: 10.1159/000324402.

[2] V. Vadori, J.-M. Graic, A. Peruffo, G. Vadori, L. Finos, and E. Grisan, "CISCA and CytoDARK0: a Cell Instance Segmentation and Classification method for histo (patho) logical image Analyses and a new, open, Nissl-stained dataset for brain cytoarchitecture studies." *arXiv preprint arXiv:2409.04175*, 2024.

[3] V. Vadori, J.-M. Graic, A. Peruffo, G. Vadori, L. Finos, and E. Grisan, "CytoDARK0", *Zenodo* (<https://doi.org/10.5281/zenodo.13694738>), 2024.

[4] L. Corain, E. Grisan, J.-M. Graic, R. Carvajal-Schiaffino, B. Cozzi, and A. Peruffo, "Multi-aspect testing and ranking inference to quantify dimorphism in the cytoarchitecture of cerebellum of male, female and intersex individuals: a model applied to bovine brains." *Brain Structure and Function*, vol. 225, no. 9, pp. 2669–2688, 2020, doi: 10.1007/s00429-020-02147-x.

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Application of Surface Measurement Techniques in Veterinary and Animal Sciences: Integrating 3D Scanning, Photogrammetry, and 3D Printing

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Advanced 3D imaging techniques, such as high-resolution scanning of organs and entire specimens, have become increasingly helpful tools to extract morphology and anatomical structures. The Department of Comparative Biomedicine and Food Science, University of Padua has recently been involved in the collection and analysis of anatomical three-dimensional (3D) data to enhance both research and educational activities. At the University's necropsy facility, when feasible, animals brought for necropsy are scanned, allowing them to be stored for a subsequent detailed study of their external anatomy and potential external lesions. These data contribute to the development of digital repositories, facilitating both research and the creation of educational materials. However, when dealing with large species, such as cetaceans, traditional 3D scanning methods often prove impractical due to logistical constraints.

To overcome these challenges, photogrammetry using Unmanned Aerial Vehicles (UAV) has been applied as an innovative solution. This approach allows for the precise acquisition of morphological data from large specimens, enabling the generation of accurate 3D models. In 2024, this method was successfully applied to three stranded cetaceans in Italy: two fin whales (*Balaenoptera physalus*) and one sperm whale (*Physeter macrocephalus*). Photogrammetry provided detailed descriptions of morphometric measurements, correlated to traditional measurements, which were further analyzed post-necropsy, ensuring data integrity even in cases where direct physical examination was not feasible. Integrating drone-based imaging with traditional scanning methods broadens the scope of morphological research, offering a complete solution framework for anatomical and related studies.

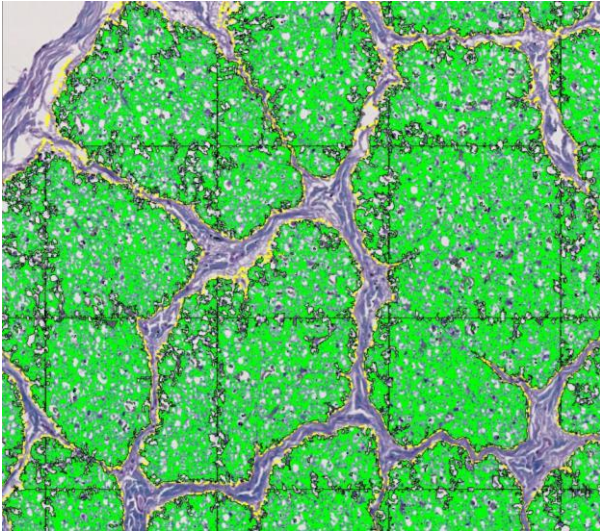
Furthermore, the digital models generated through scanning and photogrammetry can be transformed into physical scaled-up or down replicas using 3D printing technologies. A significant achievement in this field emerged during the reconstruction of the skeleton of a fin whale and two sperm whales, where 3D printing played a crucial role in replacing missing or damaged skeletal elements. These 3D-printed models, extracted either from CT scans or 3D scanning of pair parts, serve multiple purposes, including educational training, comparative anatomical studies, and museum exhibitions. In veterinary education, 3D-printed models provide a valuable resource for students, allowing them to examine anatomical structures in a hands-on manner, and can be printed at low cost on a case/need basis.

The integration of these cutting-edge measurement techniques—combining 3D scanning, photogrammetry, and 3D printing—demonstrates the advancements in veterinary and animal science research. By leveraging these technologies, researchers can overcome traditional limitations, improve educational methodologies, and contribute to a deeper understanding of anatomical structures.

Enhancing Histopathological Assessment: Automated Analysis of Bovine and Equine Optic Nerves

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The use of specific image analysis algorithms can effectively address the challenges associated with subjective assessment in veterinary pathology [1]. This is particularly important in neuropathology, where key aspects of histopathologic assessment, such as measuring nerve cell loss or the extent of glial proliferation [2], often depend on the subjective judgment of the operator. To address this issue, we focused our efforts on quantifying nerve fiber density, degree of myelination, amount of interstitial tissue, and glial cell population in normal bovine and equine optic nerves, with the goal of providing a dataset for subsequent evaluation of pathological specimens.

Optic nerves from 24 cattle (aged 2 days to 4 years) and 13 horses (aged 5 days to 30 years) regularly slaughtered or euthanized for non-neuro-ophthalmologic conditions were formalin fixed and paraffin embedded. Transverse sections

were stained with hematoxylin and eosin, Luxol fast blue (LFB), and Goldner's trichrome. Immunoperoxidase was performed on sections of the optic nerve using glial fibrillary acidic protein (GFAP), oligodendrocyte transcription factor 2 (Olig2), and phosphorylated neurofilaments (2F11) as markers for astrocytes, oligodendrocytes, and axons, respectively. All slides were scanned with the Hamamatsu NanoZoomer and analyzed with AI-driven software (Visiopharm, Hoersholm, Denmark). Optic nerve sections were selected by manually tracing regions of interest (ROIs) on whole slide images. A deep learning trained application then refined the ROIs, excluding interstitial tissue. Multiple sections per slide (ranging from 2 to 5) were analyzed individually within separate ROIs. For LFB-stained slides, another application quantified myelinated fibers by defining a threshold for blue-stained areas relative to the total area analyzed. In Goldner-stained sections, interstitial tissue was distinguished and quantified in a similar manner. 2F11-immunolabeled axons in the optic nerve were also counted separately for each ROI using the threshold method. Myelin sheath/total area (ROI) and interstitial tissue/total area (ROI) calculations were performed on 147 bovine and 129 equine optic nerve sections stained with LFB and Goldner, respectively, while the number of axons in each optic nerve was also counted in an equal number of bovine and equine sections immunolabeled with 2F11. Analysis of GFAP and Olig2 expression completed the dataset.

By providing a quantifiable dataset of the optic nerve components, our approach lays the foundation for future evaluation of pathological samples, ultimately improving diagnostic accuracy and research in neurodegenerative diseases.

We acknowledge the Center for Instrument Sharing of the University of Pisa (CISUP) for their support in image acquisition and analysis.

- [1] Zuraw A, Aeffner F. Whole-slide imaging, tissue image analysis, and artificial intelligence in veterinary pathology: An updated introduction and review. *Vet Pathol.* 2022;59(1):6-25. doi:10.1177/03009858211040484.
[2] Wilson LA, Heraty L, Ashford BA, et al. Tissue microarray (TMA) use in post mortem neuropathology. *J Neurosci Methods.* 2021;347:108963. doi:10.1016/j.jneumeth.2020.108963

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Sustainable Grass-fed supply chain: the “Filierba” case

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In recent decades, dairy farms have increasingly adopted intensive production methods, leading to the abandonment of summer grazing and traditional grasslands. This shift has caused environmental issues such as soil degradation, reforestation, biodiversity loss, and reduced landscape quality. Intensive farming also has a greater environmental impact due to the heavy use of fertilizers and pesticides.

Life Cycle Assessment (LCA) is a key tool for evaluating dairy production’s environmental impact, with studies identifying on-farm activities as major contributors. Key factors include enteric fermentation, feed production, and manure management. Research highlights that grasslands help mitigate global warming and biodiversity loss, while intensive systems have higher environmental impacts. Traditional alpine cheese production supports local resources and heritage. Full-grazing and efficient manure management can reduce GHG emissions by up to 15%. This study, part of the “Filierba” project financed by Regione Piemonte, assesses the sustainability of Piedmontese dairy farms, focusing on how feeding and breeding strategies influence their impact.

A questionnaire was distributed to Piedmontese farmers to evaluate farm sustainability. Farmers were selected based on their level of grass use in cattle diets. The survey covered farm characteristics, size, and management practices, with a particular focus on cattle diet composition. Fourteen dairy farmers participated. Data were analyzed using cluster analysis to group farms with similar land use and feeding strategies. Finally, average values were calculated for each cluster to assess the environmental impact of different production methods.

The findings revealed that many Piedmontese farms incorporate grass resources into production, offering a potential strategy to reduce dairy farming’s environmental impact. Grass use contributes to carbon sequestration and biodiversity conservation while supporting local traditions and communities. In Piedmont, grass resources are mainly found in hilly and mountainous regions, where milk production remains economically significant. Cows in these areas typically graze outdoors, with grass as a key part of their diet.

Feed self-sufficiency is another crucial factor. Among surveyed farmers, 94% produce their own hay and grass silage, while only 54% grow grains, and just 5% produce compound feed. Farms relying more on compound feed tend to have a higher environmental impact. Increasing grass in cattle diets could therefore be an effective strategy for mitigating the environmental footprint of dairy production.

This study highlights key considerations for improving dairy sustainability. While GHG emissions are not primarily driven by feed type, other environmental impacts are. Grass resources’ role in carbon sequestration and biodiversity should be considered in policymaking and farm management decisions.

Keywords: Grass-fed, environmental impact, sustainability, LCA, supply chain

1. Gusmeroli, F., Paoletti, R., Pasut, D., (2006). “Una foraggicoltura al servizio dell’allevamento e del territorio montano: tradizione e innovazione a confronto”. *Quaderno SoZooalp* 3.
2. Streifeneder, T., Tappeiner, U., Ruffini, F.V., Tappeiner, G., Hoffmann, C., 2007. Selected aspects of agro-structural change within the Alps. A comparison of harmonised agro-structural indicators on a municipal level. *Journal of Alpine Research* 3, 41-52.
3. Sturaro, E., Cocca, G., Gallo, L., Mrad, M., Ramanzin, M., (2009). “Livestock systems and farming styles in Eastern Italian Alps: an on-farm survey”. *Italian Journal of Animal Science* 8, 541-554.
4. Penati, C., Anna, S., Tamburini, A. and M. B. (2010), “Effect of farming system changes on life cycle assessment indicators for dairy farms in the Italian Alps”, *Planta Medica - PLANTA MED*, Vol. 1.
5. Fantin, V., Buttol, P., Pergreffi, R., Masoni, P. (2012). “Life cycle assessment of Italian high quality milk production. A comparison with an EPD study”. *Journal of Cleaner Production*, 28, 150–159
6. González-García, S., Castanheira, E., Dias, A. C., & Arroja, L. (2013). “Using life cycle assessment methodology to assess UHT milk production in Portugal”. *Science of the Total Environment*, 442, 225–234.
7. Guerci, M., Bava, L., Zucali, M., Tamburini, A. and Sandrucci, A. (2014), “Effect of summer grazing on carbon footprint of milk in Italian Alps: A sensitivity approach”, *Journal of Cleaner Production*, Elsevier Ltd, Vol. 73, pp. 236–244, doi: 10.1016/j.jclepro.2013.11.021.

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8. O'Brien, D., Shalloo, L., Patton, J., Buckley, F., Grainger, C. and Wallace, M. (2012), "A life cycle assessment of seasonal grass-based and confinement dairy farms", *Agricultural Systems*, Vol. 107, pp. 33–46, doi: 10.1016/j.agsy.2011.11.004.
9. Verduna, T., Blanc, S., Merlino, V.M., Cornale, P. and Battaglini, L.M. (2020), "Sustainability of Four Dairy Farming Scenarios in an Alpine Environment: The Case Study of Toma di Lanzo Cheese", *Frontiers in Veterinary Science*, Frontiers Media S.A., Vol. 7, doi: 10.3389/fvets.2020.569167.

Soil health and circularity in livestock breeding

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Soil health has recently been recognised as a prior goal to realize a sustainable agricultural production based on the circularity principles [1]. Crop rotation, use of manure or organic fertilisers, minimum or no tillage, and reduction of use of plant protection products are some of the crucial practices to increase the system sustainability, managing agricultural processes, and increasing the resilience of the agri-food sector [2–4]. European and National legislations are fostering the implementing sustainable agronomic protocols, such as agroecology, and a circular approach to the processes based on the overall life cycle of the agri-food produce [5, 6]. It is also true, that in many cases the application of these sustainable solutions, such as organic farming techniques, implies the use of expensive input with a not negligible increasing of costs for farmers [7]. In this context, livestock breeding activities could play a very important role in providing a matrix for improving organic matter and soil health in general by containing the above-mentioned costs for farmers. This paper aims to investigate the environmental implications of the use of waste from livestock breeding as soil improvers for a sustainable farming system. The life cycle methodology was used for assessing the environmental performance of the farming systems by comparing different scenarios of using soil improvers coming from livestock breeding activities and others from different organic matrixes. Data comes from the experimentations carried out during the SHARInG-MeD (GA 2211) activities. The analysis takes into consideration the phases of collecting and transportation, spreading on the agricultural land and emissions in air, soil, and water, deriving from the application of manure and organic matter. The modelling phase was carried out using the LCA software “Sphera LCA for Experts”. Results could be considered as the first phase of a more complex evaluation of the environmental performance of sustainable farming practices that are addressed to improve soil health.

Keywords: SHARInG-MeD project, Life Cycle Assessment, Soil Health, Organic Matter, Sustainable Farming, Soil Carbon Content

1. De Boer, M.I., Van Ittersum, M.K.: Circularity in agricultural production. (2018)
2. Kan, M., Amin, H.: A REVIEW ON ISSUES AFFECTING SUSTAINABLE AGRI-FOOD SUPPLY CHAIN. *Agricultural Sciences Journal*. 176–189 (2024). <https://doi.org/10.56520/asj.24.320>
3. Arimany-Serrat, N., Montanyà, O., Amat, O.: Sustainability as a Resilience Factor in the Agri-Food Supply Chain. *Sustainability*. 16, 7162 (2024). <https://doi.org/10.3390/su16167162>
4. Gaitán Cremaschi, D.: Sustainability metrics for agri-food supply chains, (2016)

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5. Farmer, A.: Developing the Circular Economy in the European Union. In: Circular Economy: Global Perspective. pp. 389–412. Springer Singapore, Singapore (2020)
6. Domenech, T., Bahn-Walkowiak, B.: Transition Towards a Resource Efficient Circular Economy in Europe: Policy Lessons From the EU and the Member States. *Ecological Economics*. 155, 7–19 (2019). <https://doi.org/10.1016/j.ecolecon.2017.11.001>
7. Reganold, J.P., Wachter, J.M.: Organic agriculture in the twenty-first century. *Nat Plants*. 2, 15221 (2016). <https://doi.org/10.1038/nplants.2015.221>

Towards sustainable mussel farming: innovative practices and perceptions of Italian operators

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Abstract

The Strategic guidelines for a more sustainable and competitive European aquaculture sector address challenges and opportunities in line with the European Green Deal and related strategies. A key priority is to reduce the environmental impact of aquaculture products by promoting the use of more sustainable technical and biological inputs under the call of the circular economy [1].

Mussel farming, one of the most important sub-sectors in Europe, is facing new challenges as it has been identified as a significant source of marine pollution [2]. Indeed, the sector extensively uses plastic materials for production and packaging [3, 4]. It is estimated that approximately 20 grams of plastic are used for each kilogram of mussels marketed [5].

Considering this, adopting biodegradable or recycled materials is a promising solution to reduce dependence on fossil-based plastics and improve industry waste management [6]. However, implementing these solutions is limited due to various obstacles [7, 8] that have not yet been adequately explored.

As part of the outcomes of the 'LIFE MUSCLES' project (LIFE20 ENV/IT/000570) [9], this study aims to investigate the economic feasibility of using biodegradable or recycled nets in Italian mussel farming. Specific objectives include (i) identifying the main barriers to adoption, (ii) exploring the perspectives of Italian mussel farmers, and (iii) offering policy recommendations in line with European strategies.

The methodology involves interviews with Italian mussel farmers to identify the main cost of alternatives to conventional plastic nets. Furthermore, economic data are considered to assess the costs and benefits of implementing sustainable materials in the sector.

The results show that, although farmers recognize the environmental benefits of alternative nets, significant barriers persist, such as high costs and doubts about long-term performance. In addition, the lack of institutional support and consumer acceptance uncertainty make the adoption process more complex.

The implications of this study highlight the urgent need for institutional support to offset initial costs, a clear regulatory framework, and awareness-raising campaigns.

[1] Commission regulation (EU) 2019/640, (2019). <https://eur-lex.europa.eu/legal-content/IT/TXT/?uri=CELEX:52019DC0640>

[2] Tudini, L., & Forgione, A. (2024). Issues and Needs for the Sustainable Development of Shellfish Farming in Italy. *Aquaculture Journal*, 4(2), 55-75. <https://doi.org/10.3390/aquacj4020005>

[3] Bottari, T., Mghili, B., Gunasekaran, K., & Mancuso, M. (2024). Impact of plastic pollution on marine biodiversity in Italy. *Water*, 16(4), 519. <https://doi.org/10.3390/w16040519>.

[4] Fortibuoni, T., Amadesi, B., & Vlachogianni, T. (2021). Composition and abundance of macrolitter along the Italian coastline: The first baseline assessment within the European Marine Strategy Framework Directive. *Environmental Pollution*, 268, 115886. <https://doi.org/10.1016/j.envpol.2020.115886>

[5] Pietrelli, L. (2022). Polypropylene recovery and recycling from mussel nets. *Polymers*, 14(17), 3469. <https://doi.org/10.3390/polym14173469>

[6] Bains, M., Fossi, M. C., Degli Innocenti, F., Chinaglia, S., Tosin, M., Pecchiari, M., & Panti, C. (2024). Degradation of plastic materials in the marine environment: A mussel farm as a case study for the development of alternative mussel nets. *Journal of Cleaner Production*, 450, 141825. <https://doi.org/10.1016/j.jclepro.2024.141825>

[7] Vecchio, Y., Masi, M., & Adinolfi, F. (2023). From the AKAP to AKAIE model to assess the uptake of technological innovations in the aquaculture sector. *Reviews in Aquaculture*, 15(2), 772-784. <https://doi.org/10.1111/raq.12756>

[8] Arantzamendi, L., Andrés, M., Basurko, O. C., & Suárez, M. J. (2023). Circular and lower impact mussel and seaweed aquaculture by a shift towards bio-based ropes. *Reviews in Aquaculture*, 15(3), 1010-1019. <https://doi.org/10.1111/raq.12816>

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[9] Life MUSCLES: Circular innovation to protect the sea. <https://lifemuscles.eu/>

ARMONIA: an Automated Remote MONitoring system of Nitrogen in Agriculture

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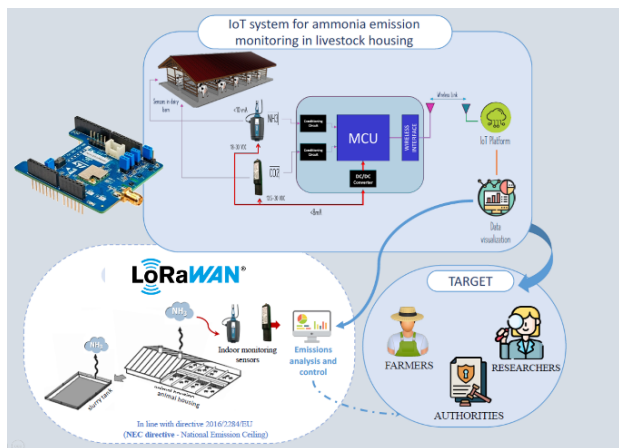


Figure 1 – IoT system for ammonia emission monitoring in livestock housing

An innovative monitoring system based on an IoT sensor network with LoRaWAN technology has been developed to monitor ammonia (NH₃) and carbon dioxide (CO₂) concentrations in buffalo barns. The system uses electrochemical DOL 53 [1] sensors for ammonia detection and DOL 119 [2] sensors for CO₂ measurement, selected for their precision, robustness, and ability to operate in challenging agricultural environments. The sensors, powered at 24V, were also chosen for their ceiling-mounting capability, a strategic position where greenhouse gases tend to accumulate, allowing for more accurate concentration monitoring. These sensors have an energy consumption of approximately 2-4W, ensuring continuous and reliable measurements.

The IoT nodes were designed using USI® STM32™ Nucleo expansion boards for LoRa™ (I-NUCLEO-LRWAN1), integrating an STM32L052T8Y6 microcontroller based on Cortex®-M0+ and a Semtech SX1272 radio transceiver. This configuration enables efficient long-range data

transmission with reduced energy consumption. The STM32L0 microcontroller consumes about 3 mA in active mode, while the SX1272 module uses around 40 mA during transmission. Both components consume approximately 1 µA in standby mode. This setup allows the node to operate with an average consumption of 0.444 W, providing an autonomy of about 33 days with a 360 Wh lithium-ion battery.

The sensor network leverages LoRaWAN technology for data transmission, ensuring stable and secure communication over distances of up to 15 km in rural environments. The nodes are enclosed in IP65-rated housings, resistant to dust, humidity, and corrosive agents, which are typical conditions in barns. The data collected is sent to a central gateway and then transmitted to a custom-developed IoT platform for data processing, management, and real-time visualization. This platform enables continuous and precise environmental monitoring, quickly identifying any anomalies or threshold exceedances.

The ultimate goal of the system is the creation of a “Digital Twin” of the farm, a virtual model that integrates real-time data to provide a dynamic and detailed representation of environmental conditions. This Digital Twin will, in the future, allow for the simulation of operational scenarios, resource management optimization, and the prediction of potential environmental issues, supporting more efficient and sustainable farm management.

This system represents a significant improvement in environmental monitoring for buffalo barns, offering a technological solution for efficient data collection and analysis. The insights gained can be used to optimize operational and environmental management, supporting informed decision-making and promoting more sustainable agricultural practices [4]. Future research will focus on enhancing system scalability, integrating additional environmental parameters, and improving predictive analytics within the Digital Twin framework.

[1] Available online at: <https://www.dol-sensors.com/products/dol-53-ammonia-sensor/>

[2] Available online at: <https://www.dol-sensors.com/products/dol-119-co2-sensor/>

[3] Wang, L. Digital Twins in Agriculture: A Review of Recent Progress and Open Issues. *Electronics* 2024, 13, 2209. <https://doi.org/10.3390/electronics13112209>.

[4] Kumar, Raman, Harpreet Kaur Channi, and Harish Kumar Banga. "Data analytics in agriculture: Predictive models and real-time decision-making." *Smart agritech: Robotics, AI, and internet of things (IoT) in agriculture* (2024): 169-200..

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Comparison between offshore and inland aquaculture from an environmental perspective

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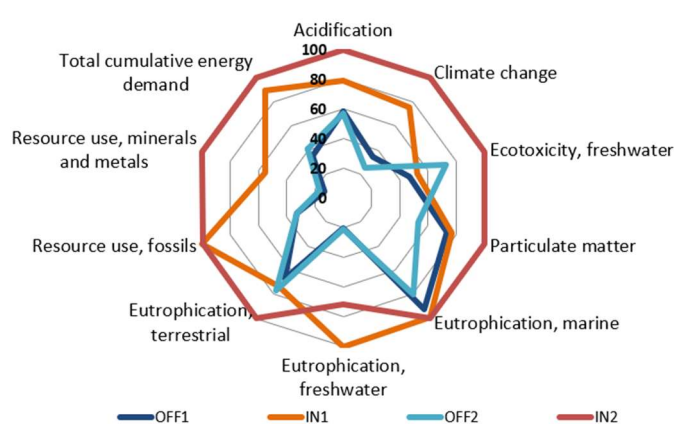


Figure 1 - Relative comparison (%) of the environmental impacts analysed across different farms

Aquaculture is a rapidly expanding sector, particularly in the Mediterranean region, where European sea bass (*Dicentrarchus labrax*) and Gilthead sea bream (*Sparus aurata*) represent key species. This study evaluates the environmental impact of different rearing systems for fish farming, comparing offshore (OFF) and inland (IN) aquaculture farms in Italy using a Life Cycle Assessment (LCA) approach. The functional unit (FU) adopted is 1 kg of fish biomass harvested, and the system boundary follows a "cradle-to-gate" perspective, including feed production, energy use, and farm operations.

Primary data were collected directly from farms, including feed composition, energy consumption, fish growth rates, and infrastructure characteristics. Secondary data were sourced from relevant databases (e.g., Ecoinvent 3.8) and literature to model background processes such as electricity production and feed ingredient processing. The

estimation of nitrogen (N) and phosphorus (P) emissions from fish metabolism was performed using a mass balance approach, following established nutrient retention models [1]. The Environmental Footprint 3.1 method was applied for impact characterization.

Results indicate that inland systems exhibit significantly higher environmental burdens compared to offshore systems, particularly in Climate Change (CC) and resource use. Climate Change impact for offshore farms ranges from 2.37 to 3.24 kg CO₂ eq/kg fish, whereas inland farms reach 7.08 to 9.40 kg CO₂ eq/kg fish. This difference is largely attributed to high energy demands in inland facilities, primarily for water pumping and oxygenation, which contribute to increased fossil fuel consumption. Eutrophication impacts show mixed trends, with terrestrial eutrophication ranging from 0.0996 to 0.1154 mol N eq for offshore and up to 0.1494 mol N eq for inland farms. Freshwater eutrophication is particularly high in inland farms, reflecting nutrient discharges from intensive production. The contribution analysis highlights that feed production dominates most impact categories, particularly in offshore systems, where it accounts for over 80% of CC impact. In contrast, in inland systems, energy consumption contributes up to 40% of CC, with oxygenation processes playing a significant role.

These findings align with previous studies highlighting the environmental trade-offs between different aquaculture systems [1] [2]. Further research should focus on optimizing feed composition and management practices to minimize emissions and resource use.

[1] Bureau, D. P., & Hua, K. (2010). Towards effective nutritional management of waste outputs in aquaculture, with particular reference to salmonid aquaculture operations. *Aquaculture Research*, 41(5), 777-792.

[2] Ayer, N., Martin, S., Dwyer, R. L., & Laurin, L. (2016). Environmental performance of copper-alloy net-pens: Life cycle assessment of Atlantic salmon grow-out. *Aquaculture*, 453, 93-103.

[3] Bohnes, F. A., Hauschild, M. Z., Schlundt, J., & Laurent, A. (2019). Life cycle assessments of aquaculture systems: A critical review of reported findings with recommendations for policy and system development. *Reviews in Aquaculture*, 11(4), 1061-1079.

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Smart monitoring and control of the environmental conditions of a swine nursery barn for fossil-free heating

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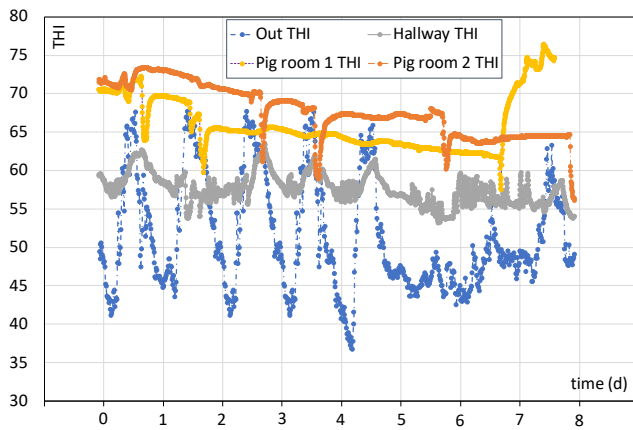


Figure 1 - THI measured in the heated and monitored indoor spaces of the nursery barn and outside.

Livestock farms are characterized by significant energy demands, particularly for operating equipment and maintaining optimal climate conditions in animal housing. In fact, climate control is increasingly crucial due to the growing emphasis on animal welfare, also related to high production standards in terms of quantity, quality, and food safety. At the same time, the sector presents significant opportunities for renewable energy production and utilization, leveraging available spaces on building roofs and open courtyards [1].

This study presents the development and implementation of a fossil-free heating system designed for a farrow-to-nursery swine farm with 500 sows in the Po Valley, Italy. The system integrates solar thermal and photovoltaic energy with geothermal storage to meet the heating requirements of the farm's nursery building [2]. Additionally, a smart monitoring system was incorporated to track key environmental and energy parameters, serving

as both a data collection tool and an automated control system for the equipment. Based on site-related parameters, this integrated system was specifically designed to support swine farm heating needs during colder seasons and it was implemented as part of the European project RES4LIVE [3], with a particular focus on sustaining optimal conditions for weaning pigs.

The study analyzed the heating system's performance during a period of significantly low external temperatures, averaging 9.2°C with a minimum of 2.9°C, necessitating continuous heating. The environmental data collected via the smart monitoring system confirmed the system's effectiveness in maintaining appropriate temperature and humidity levels. The results demonstrated that the system effectively maintained the indoor Temperature-Humidity Index (THI) below the alert threshold of 75 [4] throughout the study period (Figure 1). The findings underscored the feasibility of integrating multiple renewable energy components into an automated heating system tailored to livestock farming. By leveraging solar energy, thermal storage, and a heat pump, the system efficiently managed indoor climate conditions without relying on fossil fuels.

The results highlight the potential of renewable energy solutions to enhance sustainability in livestock farming while ensuring animal welfare and operational efficiency. Future research will focus on expanding this approach to further farm building typologies related to the study case, such as farrowing barns, while also integrating solar and geothermal energy resources to regulate temperature and humidity in hot weather conditions.

- [1] S. Hosouli *et al.*, "Evaluation of a solar photovoltaic thermal (PVT) system in a dairy farm in Germany," *Solar Energy Advances*, vol. 3, p. 100035, 2023, doi: <https://doi.org/10.1016/j.seja.2023.100035>.
- [2] D. Murali *et al.*, "Experimental assessment of a solar photovoltaic-thermal system in a livestock farm in Italy," *Solar Energy Advances*, vol. 4, p. 100051, 2024, doi: <https://doi.org/10.1016/j.seja.2024.100051>.
- [3] D. Tyrís *et al.*, "RES4LIVE – Energy Smart Livestock Farming towards Zero Fossil Fuel Consumption," *VDI Berichte*, vol. 2022, no. 2406, pp. 493–498, 2022, doi: [10.51202/9783181024065-493](https://doi.org/10.51202/9783181024065-493).
- [4] H. Shin, S. Lee, J. Kim, D.-H. Park, S.-K. Jo, and Y. Kwak, "Applicability evaluation of a temperature humidity index-controlled ventilation system in livestock using a building energy simulation model," *Case Studies in Thermal Engineering*, vol. 57, p. 104335, 2024, doi: <https://doi.org/10.1016/j.csite.2024.104335>.

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Do the Largemouth bass like live insects? A preliminary study with three insect species as feed enrichment

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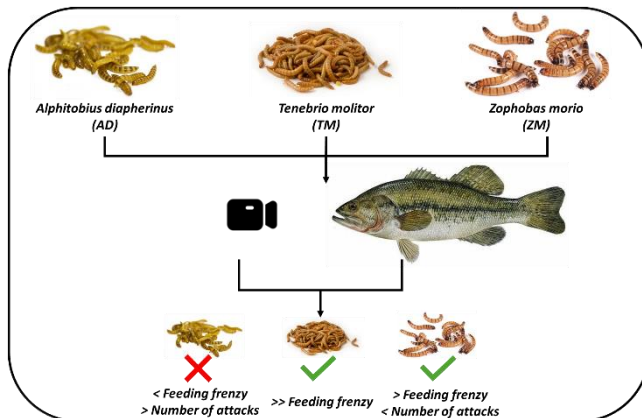


Figure 1 – Graphical representation of the experimental trial and main results obtained.

Aquaculture has experienced significant growth in recent years, and this trend is expected to continue. A major challenge facing the sector is the need to find cost-effective and nutritionally suitable feeds. In September 2023, the Italian Ministry of Health authorized the use of live insects as feed for fish, poultry, and pigs within Italy. This study aims to analyse the effects of feed enrichment with live insects on the behaviour and production performance of Largemouth bass (*Micropterus salmoides*). The experiment was conducted at the aquaponics facility of the University of Padua (DAFNAE), where three types of Coleopteran larvae were used: *Alphitobius diaperinus*, *Tenebrio molitor*, and *Zophobas morio*. The trial lasted for 28 days and involved 157 fish distributed across 9 tanks. The larvae were administered to each tank at a rate of 10% of the average daily intake.

Fish were monitored for health and growth performance. The behavioural repertoire was observed indirectly, using a video recording system (Figure 1). Although the results indicated no significant variations in growth metrics—such as growth rate, average daily growth, average daily consumption, and growth index—behavioural variables were affected by the inclusion of insects in their diet. When the fish were fed pellets, they consumed them more quickly and displayed intense and prolonged feeding frenzy behaviour compared to when they were fed live larvae. Specifically, fish fed with *Alphitobius diaperinus* larvae exhibited shorter feeding frenzy durations but a higher number of attempts and attacks on the food resource, leading to longer total feeding times and consequently slower feeding rates. *Tenebrio molitor* larvae resulted in the longest feeding frenzy durations, while *Zophobas morio* larvae recorded the fewest attacks, along with the shortest total feeding time (Figure 1). Currently, *Zophobas morio* is not an insect species approved for use as feed, but these preliminary results suggest that it could play a significant role in fish nutrition, particularly as a form of dietary enrichment.

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Antimicrobial resistance and virulence genes in *Escherichia coli* isolated from young healthy chickens

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Escherichia coli is a gram-negative bacterium commonly found as a commensal in the gastrointestinal tract of humans and many animals, including domestic poultry [1]. It is frequently associated with multiple antimicrobial resistances and is a frequent cause of intestinal and extra-intestinal infections [2, 3].

The main intestinal pathotypes include Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC), Enteroaggregative *E. coli* (EAEC), and Necrotoxicogenic *E. coli* (NTEC). Each pathotype is characterized by distinct virulence factors and mechanisms of pathogenicity [4, 5].

The present study aimed to investigate the phenotypic and genotypic antimicrobial resistance, and to detect the presence of virulence factors in *E. coli* isolated from 120 young, healthy chickens never treated with antibiotics and coccidiostats. During the summer seasons of 2022 and 2023, 120 cloacal swab samples were collected and submitted to bacteriological cultures, and one *E. coli* strain was selected for each sample.

The disk diffusion method employed on all isolates revealed the highest resistance to chloramphenicol and trimethoprim-sulfamethoxazole, both at 11.67%, followed by ampicillin and tetracycline at 10% each. In terms of susceptibility, aztreonam and gentamicin showed the highest percentages, with 100% of isolates being susceptible. Amikacin had 99.17%, while cefoxitin, ciprofloxacin, and ertapenem each had 98.33% susceptibility. For intermediate resistance, cefotaxime had the highest percentage with 32.5%, followed by imipenem with 13.33%, ampicillin with 9.17% and ceftiofur with 6.67%. Based on these results, eight strains (8/120, 6.67%) were found to be multidrug-resistant (MDR) [6].

Molecular analyses conducted on 13/120 (10.83%) phenotypically penicillin-resistant strains showed that 11/13 (84.62%) had the *bla*_{TEM} gene. The *astA* gene was found in 18/120 (15%) isolates, potentially indicative of the EAEC pathotype, of which 5 were MDR and 4 had both the *astA* and *bla*_{TEM} genes. Virulence genes characterizing other pathotypes were not detected.

The finding of *E. coli* strains with resistance and/or intermediate resistance to different antimicrobials shows that chickens reared in optimal conditions and never treated with antibiotics may harbor bacterial strains that, by contaminating eggs and meat, could become a public health hazard. In addition, strains with resistance genes can act as donors of this genetic material to other bacteria. Poultry farm hygiene is fundamental to reduce the circulation of pathogenic bacteria in the environment.

1. Stromberg, Z. R. et al., Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health. *PLoS one*, vol 12(7), e0180599, 2017.
2. Mellata M., Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Food-borne pathog dis*, vol 10(11), 2013.
3. Sarowska, J. et al., Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. *Gut pathog.*, vol 11, pp. 10, 2019.
4. Kaper, J. B., Nataro, J. P., & Mobley, H. L., Pathogenic *Escherichia coli*. *Nat. rev. Microbiol.*, vol 2(2), pp. 123–140, 2004.
5. Pokharel, P., Dhakal, S., & Dozois, C. M., The Diversity of *Escherichia coli* Pathotypes and Vaccination Strategies against This Versatile Bacterial Pathogen. *Microorganisms*, vol 11(2), pp. 344, 2023.
6. Magiorakos, A. P et al., Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin microbiol infect*, vol 18(3), pp. 268–281, 2012.

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Application of digital technologies to promote coexistence between wolves and sheep farms

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Mitigating conflicts associated with predation on livestock is crucial for conserving large carnivores in human dominated landscape [1;2;3]. To achieve this purpose, old methods of the ancient pastoral tradition (such as guardian dogs) and new technologies, have become desirable to promote coexistence between livestock husbandry and wolves.

In this context, the BELA project was proposed by the Department of Veterinary Sciences and accepted within the PR-Toscana, ESF+2021/2027 (European Social Found). The project takes place in an anthropized rural territory, outside protected areas, subjected to the recent and documented return of the wolf [4]. This landscape is characterized by recreational and hospitality activities and therefore pastoralism does not involve the use of prevention methods such as anti-wolf fences and guardian dogs.

The project involves the conception, design and realization of new digital device for monitoring the main physiological parameters of sheep and their vocal emissions aimed at promoting coexistence between farms and wolves.

The device consists in a collar integrated with individual sensors (sound and accelerometer recorder) and sensors for physiological parameters (heart rate and temperature). The information relating to the sheep and any change in the recorded parameters will be sent, via a gateway positioned in the pasture, directly to the shepherd's mobile phone or to some dissuasion mechanisms. The aim of the project is to investigate which physiological parameters or set of auditory and behavioural data can be used to develop an early warning of a wolf attack and the consequent activation of deterrent measures.

[1] A. Gazzola, C. Capitani, L. Mattioli, and M. Apollonio, "Livestock damage and wolf presence", *Journal of Zoology*, 274: 261–269, 2008.

[2] Y. Iliopoulos, S. Sgardelis, V. Koutis, and D. Savaris, "Wolf depredation on livestock in central Greece", *Acta Theriologica*, 54: 11–22, 2009.

[3] C. Imbert, R. Caniglia, E. Fabbri, P. Milanesi, E. Randi, M. Serafini, E. Torretta, and A. Meriggi, "Why do wolves eat livestock? factors influencing wolf diet in northern Italy", *Biological Conservation*, 195: 156-168, 2016.

[4] F. Coppola, S. Baldanti, A. Di Rosso, G. Vecchio, L. Casini, C. Russo, V. Lucchini, C.B. Boni, M. Malasoma, C. Gabbani, and A. Felicioli, "Settlement of a stable wolf pack in a highly anthropic area of pisan hills: Relationship with animal husbandry and hunting in a human-wolf coexistence perspective", *Animal Science Journal*, 93(1), e13799, 2022.

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Potential application of precision technology to monitor welfare and interactions of cage-farmed fish with wild fish species

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During the recent years, the concept of sustainability in aquaculture has increased its popularity and interest in it. In this background, especially for the marine cages, the interactions between fish reared and wild fish can affect welfare and health status of both farmed and wild fish populations in the ecosystem [1]. The presence of farmed fish in cages can increase competition for food between wild and farmed fish, as they share the same marine environment. The presence of fish-eating birds and/or pelagic fish predators, as tuna and bluefish, can cause stress and damage to farmed fish populations [2,3]. Good management practices and environmental control are needed to monitor these interactions. Fish farmers are looking for reducing negative impacts through cage placement, waste management and fish welfare monitoring.

In this scenario, this study is performed to evaluate growth performance and welfare status of reared fish in off-shore cages and the possible interactions with wild fish, observed in aggregation near the cages. For the aim of the study, a non-invasive device, a precision technology system of video cameras and water quality sensors, is employed in an off-shore cage aquaculture plant, located in the Gulf of Manfredonia (Puglia region), specialized in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) farming, according to the IMTA (Integrated Multi-Trophic Aquaculture) system. This precision technology system is installed thanks to FishVision research project, supported by Agritech. This grant is equipping two farming cages (1/sea bream and 1/sea bass).



Figure 1 - Video monitoring system - Position of the 3 underwater cameras on the monitoring bracket in the farming cage.

The video monitoring consists in three cameras positioned inside the cage. Two stereoscopic cameras are synchronized for 3D analysis and another camera is placed in the opposite direction of the others, towards the outside of the net cage (Figure 1). The video streams are 10 sec long and with 3 frames; consequently, they are stored in HEVC (high-efficiency video encoding) files format, to be sent via network to a repository. A thermal camera is pointed towards the farm area to record marine currents. Water quality is monitored by means of sensors to determine the most important physico-chemical parameters. A platform connected to the repository is created to collect data. Zoo-technical performances and histological liver status are evaluated to show physio-pathological patterns and behaviour of farmed fish in presence of wild fish, in relation to farming technique and water quality trend. Based on this study, precision digital system allows to monitor the behaviour of reared fish in presence of wild fish and helps the fish farmers to predict the measures to take in order to manage this problematic interaction.

- [1] K. Toledo-Guedes, J. Atalah, D. Izquierdo-Gomez, I. Uglem, P. Sanchez-Jerez, P. Arechavala-Lopez and T. Dempster, "Domesticating the wild through escapees of two iconic mediterranean farmed fish species", *Sci. Rep.*, 14, 23772, 2024.
- [2] B. Díaz López "Temporal variability in predator presence around a fin fish farm in the Northwestern Mediterranean Sea", *Mar. Ecol.*, 38: e12378, 2017.
- [3] F. Aguado-Giménez, S. Eguía-Martínez, J. Cerezo-Valverde and B. García-García, "Spatio-temporal variability of ichthyophagous bird assemblage around western Mediterranean open-sea cage fish farms", *Mar. Environ. Res.*, vol. 140, pp. 126-134, 2018.

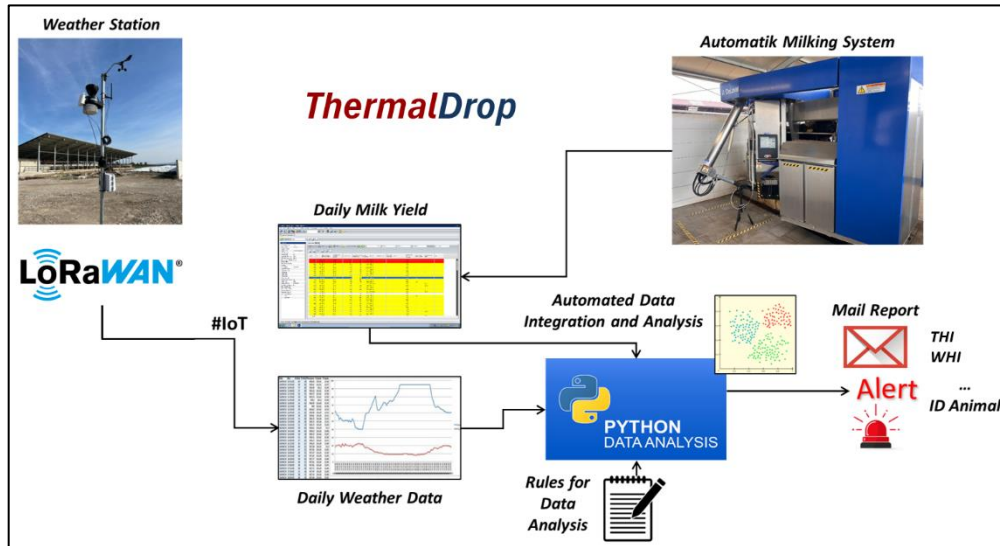
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Development and Validation of a Multi-Source Decision Support System for Cold Stress Management in Dairy Buffalo Farming, Including IoT Sensors and Milking Robot Data

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The increasing frequency of extreme weather events due to climate change poses challenges for dairy buffalo farming, potentially compromising milk production. While heat stress in dairy cows has been extensively studied, the impact of cold stress on lactating buffaloes remains largely unexplored. This study aimed to develop and validate an automated alert system to identify

Figure 1 – Scheme of Alert generation

environmental stressors and enable timely interventions. Meteorological data were continuously recorded using a Davis Pro 2 weather

station positioned near the barn, measuring temperature, humidity, wind speed and direction, every 15 minutes. This data was transmitted to a gateway, which processed and forwarded it to a centralised database for structured storage and easy access. Simultaneously, daily milk production was monitored via a DeLaval milking robot (VMS 300), which automatically generated detailed animal-specific reports. A custom Python-based software integrated these datasets, ensuring a comprehensive analysis of environmental and production factors. To assess cold stress, the software calculated two key indices: the Temperature-Humidity Index (THI), calculated as $THI = (1.8T + 32) - [(0.55 - 0.0055H)(1.8T - 26)]$, and the Windchill Index (WHI), defined as $WHI = 33 - (33 - T)(0.47 + 0.45\sqrt{Wind\ speed} - 0.05\ Wind\ speed)$. THI was derived from daily minimum temperature and maximum relative humidity, while WHI incorporated wind speed and temperature effects. Additionally, negative thermal excursions were evaluated by calculating drops in maximum temperature between consecutive days. The system automatically triggered alerts when THI fell below 58, or WHI exceeded 14 m/s, or negative temperature fluctuations surpassed 5°C. These thresholds were defined using a mixed linear model, linking daily milk production variations to climatic indices and animal characteristics such as parity, days in milk, and month variability. Alerts were sent via email, allowing farm operators to promptly adjust environmental and management conditions. Daily milk yield declined one day after alert activation, confirming the system's effectiveness in detecting cold stress. A consistent proportion of the animals (40-70%) experienced a significant daily milk reduction (-41.5% on average), while others remained unaffected. This technology represents an important step in livestock digitalisation, offering a proactive, data-driven approach to mitigate cold stress. By integrating real-time environmental monitoring with production data, it may improve buffalo welfare and farm sustainability, particularly in temperate regions where cold stress is often underestimated. Future research incorporating multi-year datasets will refine predictive models, further strengthening dairy buffalo farming's resilience to climate variability.

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Enhancing Dairy Goat Health Monitoring: Thermal Imaging for Heat Stress Detection and Behavioural Insights

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Infrared thermography (IRT) is an innovative, non-invasive tool for assessing physiological responses in livestock, particularly through the monitoring of superficial skin temperature (SST). By detecting infrared radiation emitted from the body surface, IRT provides valuable insights into thermoregulation, stress responses, and overall animal welfare [1]. While extensively studied in cattle, pigs, and sheep, the application of IRT in goats remains underexplored. The limited studies on the application of infrared thermography (IRT) in goats are further compounded by the scarcity of research investigating heat stress and its effects in this species. This gap in knowledge stems from the common assumption that, due to their hardy and adaptable nature, goats are less susceptible to the adverse effects of heat stress compared to other livestock species. However, climate change, with increasingly frequent and prolonged heat waves, poses a significant threat to goats reproductive and productive performances.

This study aimed to evaluate the effectiveness of IRT in assessing thermal stress and anticipating behavioural responses in dairy goats under severe heat stress. We monitored 18 Alpine dairy goats over six days (July 19 – August 9, 2023) during their two daily meals (7 AM and 7 PM) in their three pens to determine the relationship between their SST measured with IRT and their behaviour in different conditions of heat stress (i.e., mild, moderate, and severe). The severity of heat stress was established based on the Temperature-Humidity Index (THI) equation proposed by National Research Council [2], and heat stress was considered mild with a THI < 75, moderate with a THI comprised between 75 and 80, and severe with a THI > 80. Goats were videorecorded for 10 minutes at 7 AM and 7 PM over the six days, and their SST was then assessed using a portable thermal camera (FLIR E76 24; FLIR Systems AB, Danderyd, Sweden) and taking pictures at the same distance of 80 cm from the body region of interest. In total, five body regions were assessed, comprising the left eye lacrimal caruncle, the left flank, the loins, and the front knees and distal part of the legs. Videorecordings were then analysed by a trained observer using an ad libitum sampling method. The mutually exclusive behaviours included in the used ethogram were walking, standing, sternal recumbency, lateral recumbency, feeding, drinking, ruminating while standing, and ruminating while lying.

Among the various body regions analysed, the distal leg SST showed the strongest association with variations in environmental parameters [3]. Changes in mutually exclusive behaviours were analysed using generalized linear models with a Poisson distribution in the R environment. The behaviour most notably affected by heat stress was rumination while standing, which decreased from 23%, 22%, and 16% of total observation time under mild heat stress to just 1%, 0%, and 0% under severe heat stress in pens 1, 2, and 3, respectively. Additionally, variations in distal leg SST appeared to be potential predictors of behavioural changes. Under mild heat stress, the average SST of the distal leg was 25.8°C, with goats spending approximately 20% of the time ruminating. However, when the average distal leg SST increased by approximately 5°C (exceeding 30°C in moderate and severe heat stress), rumination time dropped to less than 2%. Overall, this study highlights the potential of IRT as a reliable and non-invasive method for early heat stress detection in dairy goats. Integrating IRT into precision livestock management systems could enhance on-farm welfare monitoring, allowing for timely interventions to mitigate heat stress and improve animal well-being and productivity. Acknowledgements: This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

[1] M.D. Ghezzi, F. Napolitano, A. Casas-Alvarado, I. Hernández-Ávalos, A. Domínguez-Oliva, A. Olmos-Hernández, A.M.F. Pereira, "Utilization of Infrared Thermography in Assessing Thermal Responses of Farm Animals under Heat Stress", *Animals*, 14(4), 616, 2024.

[2] National Research Council, "A Guide to Environmental Research on Animals", *Natl. Acad. Sci.*, Washington, DC, 1971.

[3] N.T. Masebo, M. Bolcato, M. Zappaterra, V. Bocchi, B. Padalino, L. Nanni Costa, "Identification of the best body regions for measuring superficial skin temperature (SST) in dairy goats exposed to thermal stress", 11th European Conference on Precision Livestock Farming, pp. 1194 – 1201, 2024.

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Evaluating welfare, milk quality and yield of Rendena cows in loose vs. tied housing systems

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The Rendena is a local dual-purpose Alpine cattle breed farmed in the mountain region of north-eastern Italy. It is known for its medium-small size, adaptability to pasture, and strong functional traits, particularly longevity and fertility [1]. In 2023, the Rendena population comprised 3,621 cows across 182 herds monitored for milk production and quality traits [2], with Veneto (46%) and Trentino Alto Adige (22%) hosting most of the animals [2]. Animal welfare is a fundamental aspect of dairy cow farming, helping to prevent diseases, reduce antimicrobial use, and improve milk production [4]. In Italy, the ClassyFarm system was proposed as a tool to calculate an overall herd welfare score following a dedicated farm inspection, evaluating multiple aspects such as biosecurity, management, structures, and animal-based measures [4]. Dairy farms are assessed using specific checklists for either loose housing systems (LHS) or tied housing systems (THS). The data are processed by an algorithm generating an overall welfare score ranging from 0% (poor welfare) to 100% (excellent welfare) [4].

This study aimed to assess the effect of housing systems (LHS vs. THS) on individual milk yield and quality traits in Rendena cows and to evaluate whether the ClassyFarm welfare score differs between the two systems. The dataset included 3,761 individual milk samples from 750 Rendena cows, collected between August 2022 and November 2023 from 17 single-breed herds of the Veneto region. Only test-day records from cows within 200 days before or after the ClassyFarm assessment were considered to ensure welfare evaluation simultaneity. Available data included days in milk (DIM), parity, and milk yield (kg/day), as well as fat, protein, casein, and lactose content (%), SCC (cells/mL), DSCC (%), and urea concentration (mg/dL). A linear mixed model was used for variance analysis, with housing system, DIM, and parity as fixed effects, while cow, herd-test-day, and residuals were included as random factors.

Overall, milk quality did not differ markedly between the two housing systems, as only a few traits were significantly affected. Milk yield, protein, and casein content were higher in LHS, while urea concentration was lower in LHS. Herds exhibited good welfare levels (range: 69–87%), with average scores of 80.40 % in LHS and 78.40 % in THS, showing no significant differences between the two systems. These findings suggest that, while milk quality and yield are slightly higher in LHS, welfare levels in Rendena farms did not significantly differ between LHS and THS.

[1] R. Mantovani, L. Gallo, P. Carnier, M. Cassandro, and G. Bittante, "The use of a juvenile selection scheme for genetic improvement of small populations: the example of Rendena breed," *In Proceeding of the 48th Annual Meeting of EAAP*, vol. 48, pp. 22-22, 1997.

[2] Italian Breeders Association (AIA), "BollettinoOnline Controlli sulla Produttività del Latte," 2023.

[3] A. Varotto, M. De Marchi, M. Penasa, and M. Cassandro, "A comparison of milk clotting characteristics and quality traits of Rendena and Holstein-Friesian cows". *Ital. J. Anim. Sci.*, vol. 14, 3768, 2015.

[4] G. Ventura, V. Lorenzi, F. Mazza, G. A. Clemente, C. Iacomino, L. Bertocchi, and F. Fusi, "Best farming practices for the welfare of dairy cows, heifers and calves". *Animals*, vol. 11, 2645, 2021.

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Dolfake : An open-source 3d printed dolphin allowing the standardization of stranding data collection

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Determining the causes of cetacean mortality requires standardized stranding management within the study area. However, educating scientists and volunteers on appropriate procedures is challenging due to the lack of suitable educational materials. As a niche market, there are limited training models available, which are often expensive, and not adaptable to all required scientific actions.

Considering that strandings occur worldwide, we have developed the *Dolfake* : an open-source, decentralized, 3D-printed training dolphin designed for local production near its intended area of use.



Figure 1 – First 1:1 (184cm long) 3D printed dolphin produced as a proof of concept.

By integrating various techniques such as CT scanning, photogrammetry and basic 3D design, we successfully produced a life-sized dolphin model (length : 184 cm) using a consumer-grade 3D printer (Prusa XL).

The 6 kg PLA model was divided into 10 pieces using MeshInspector 2.4.7 and sliced with PrusaSlicer 2.8.0.

Total print time is 140 hours with a total electricity consumption of 20 kWh.

The final product is a cost-effective mannequin (under 200 euros for a full-sized dolphin) enabling different teams to standardize their processes for photographing, measuring, and sampling.

Since the 3D printer market is highly diversified, the final files will include variants tailored for smaller and cheaper printers. However, the printing time, handling, and amount of material used will be higher. The main advantage of a multi-part structure is the ability to improve or fix the model piece by piece, without having to redo everything. This approach is both faster and more cost-effective.

The Dolfake is designed to be a key component of a standardized training platform including a mobile app and dedicated courses about marine mammals stranding management.

Our future objective is to develop a more sophisticated model that enables comprehensive internal examinations, particularly for training in advanced procedures, such as rib sectioning, and extraction of teeth, eyes, and inner ears. Currently three prototypes are being tested in France, Belgium, and Morocco.

Any additional collaborators interested in joining the project are welcome.

From Wildlife to Domestic: How New Technologies Can Help Veterinary and Animal Sciences

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Wildlife tracking commenced several years ago with the development of the first VHF tags. However, it has significantly advanced as a science in recent years with the advent of more sophisticated and miniaturised devices. The challenges associated with capturing and tagging wild animals have driven the creation of increasingly smaller devices with lower power consumption, greater reliability, and an expanding array of sensors, such as accelerometers and heart-rate monitors. Miniaturised accelerometers and GPS units can aid in monitoring the movements and determining the behaviour and health of individual animals.

Proximity sensors not only help in understanding animal social networks but can also detect the presence of animals near specific features, such as feeders or resting areas, similar to long-range RFIDs.

Most of these devices generate raw data, which is more challenging to analyse compared to the "ready-to-visualise" tags developed in recent years. However, raw data can uncover new and unexpected insights that pre-processed data cannot provide. In this contribution, we present findings from various studies using GPS and accelerometers on different species. Additionally, we explore how proximity sensors can be utilised in monitoring domestic animals and discuss their limitations.

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Exogenous melatonin alters nocturnal and diurnal locomotor activity, body temperature, heart rate, and heart rate variability in lambs, measured by triaxial accelerometers and subcutaneous bio-loggers.

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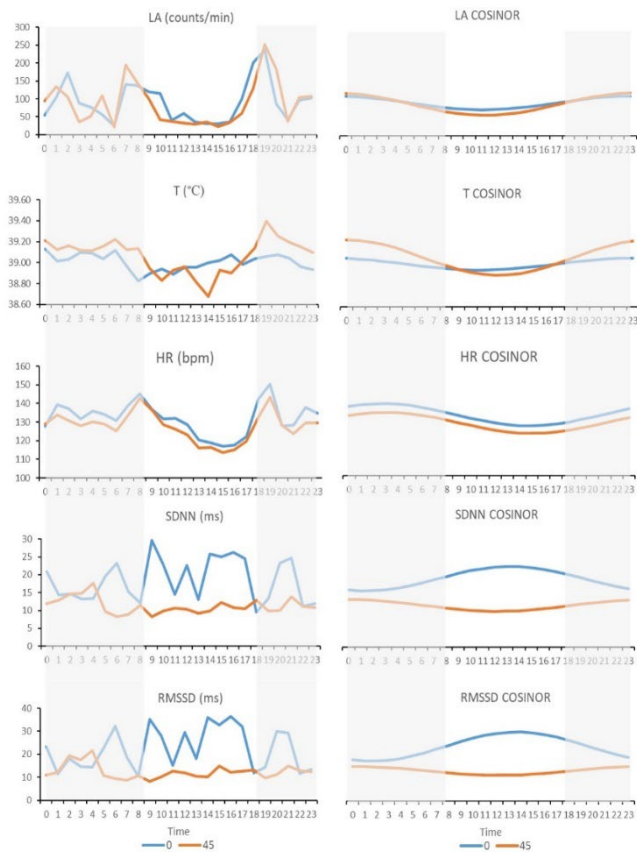


Figure 1 – Locomotor activity (LA), body temperature (T), heart rate (HR), and heart rate variability (SDNN and RMSSD) of lambs at 75 d of age, treated (group 45) or not (group 0) with melatonin at 45 d of age (grey areas indicated nighttime).

mean LA or T, but melatonin-treated lambs had lower HR, SDNN and RMSSD ($P < 0.001$) than non-treated lambs. Treated lambs had a lower LA and T, and a higher HR, during the night ($P < 0.001$), compared with daytime values. All the studied variables presented 24-h circadian rhythms. In conclusion, both triaxial accelerometers and subcutaneous bio-loggers are excellent tools to detect changes in behavior and physiology in the ovine species, caused by external factors.

[1] Molcan L (2019). *BioRxiv*, 805960.

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Smart Farming in Aquaculture: Using Biologgers to Improve Welfare and Yield

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With the rising global population, aquaculture plays an essential role in meeting the growing demand for animal protein. However, optimizing production is not just a matter of scale—animal welfare is a key factor influencing both the quality and quantity of yield. Poor welfare, driven by environmental stressors and husbandry practices, can lead to higher mortality rates, reduced growth efficiency, and increased disease susceptibility. Conversely, improving welfare conditions enhances resilience, growth rates, and overall sustainability.

To achieve this, we must accurately assess both acute and chronic stress in farmed fish. Implantable biologgers offer a powerful solution by continuously monitoring key physiological variables such as heart rate, body temperature, and activity levels. These data provide real-time insights into how fish respond to environmental changes, handling procedures, and stocking densities, allowing for science-based welfare interventions.

Here we present how biologgers can revolutionize aquaculture by providing objective, continuous welfare metrics, ultimately leading to more productive and sustainable farming practices. By integrating physiological monitoring into aquaculture management, we can move toward a future where high yields and high welfare go hand in hand.

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Effect of aquarium environmental enrichment in welfare of ornamental fish (*Poecilia reticulata*)

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The ornamental freshwater fish breeding and trade industry has seen a significant increase in recent years, with an annual value of 15 to 30 billion dollars: around 4500 species, and the 2 billion fish transported annually for hobbyists' tanks. In Italy, the ornamental fish market is regulated by law n.157/1992, that unfortunately gives only general and not species-specific monitoring protocols. Fish are sentient animals and experience different states of welfare [1]. Stress prevention is thus the cheapest and easiest way to raise healthy fish [2]. As for other animals, in ornamental fish, environmental enrichment (EE), together with parameters such as water quality, photoperiod or tank size, are essential to maintain welfare [3]. In the last ten years, the guppy (*Poecilia reticulata*) has received great interest both as ornamental fish and animal model [4]. Thus, the objective of this study was to evaluate how different levels of EE influence the welfare of guppies, through minimally invasive analyses, such as behavioral and water cortisol concentration (W_CorT) of aquarium. Three different groups (15 tanks: 5 for each group, tank size = 60L) on the basis of EE were equipped as follow: absence (A); Low level (L) on the bottom of the tank 30 mm of substrate (Amtra Ivory white quartz, 1-3mm) and one oak wood (25-30cm); high level (H), same L's tank substrate and wood on the bottom, plus two different ornamental plants, selected to recreate guppy natural environment (*Myriophyllum aquaticum* and *Pistia stratiotes*). 330 guppies were selected for homogeneous size and age and divided in the 15 experimental tanks. Fish were video recorded and W_CorT of each group were weekly evaluated. Behavioral and W_CorT data were analyzed with R for EE effect, and the significance was set at $P < 0.05$. In H group, behaviors such as “wood, flotation and plant feeding” and “courting”, which are indicators of a good quality of life, were significantly more expressed. Also “bite” was also more expressed in H group, as territorial behavior. Stereotyped behaviors, stress indexes, were significantly more expressed in A and L groups. Accordingly, W_CorT was found significantly higher in A and L tanks. It is therefore evident that the absence or low level of environmental enrichments in tanks and aquariums enable guppy to express their species-specific behavioral repertoire and improve the stress indexes. In the light of these results, we conclude that high EE levels ensure a better welfare status in guppy breeding.

[1] C. Stevens, M. Fiddes and P. Rose, “The behavioural biology of freshwater fishes.” In *The Behavioural Biology of Zoo Animals*, pp. 237-252, CRC Press, 2022.

[2] S. Magada and T.A. Mercy, Health management in ornamental fish farming. Best management practices for freshwater ornamental fish production. National Fisheries Development Board, Ministry of Agriculture and Farmer's Welfare, GOI, Hyderabadan, India, pp. 94-106, 2016.

[3] C.H. Stevens, D.P. Croft, G.C. Paull, and C.R. Tyler, Stress and welfare in ornamental fishes: what can be learned from aquaculture?. *J. Fish Biol.*, vol. 91(2), pp. 409-428, 2017.

[4] A. Deacon, ‘*Poecilia reticulata* (guppy)’, CABI Compendium. CABI International, pp. 1–20, 2023.

Cardiac Activity of horses during training measured with external Polar sensors.

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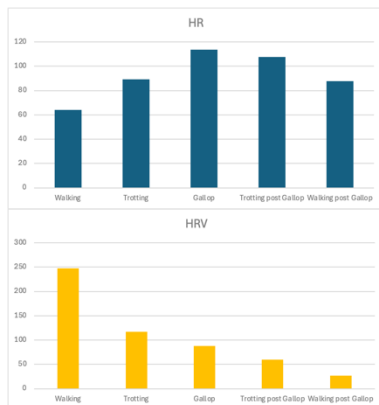


Figure 3: Heart rate (HR, bpm) and heart rate variability (HRV, ms) records as a function of the different training phases.

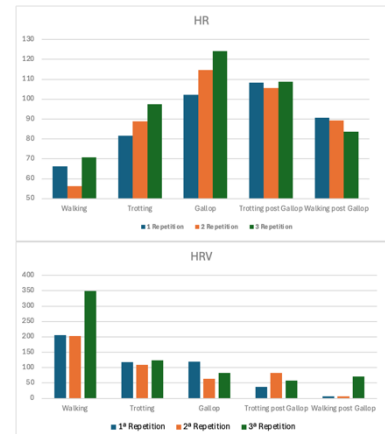


Figure 2: Heart rate (HR, bpm) and heart rate variability (HRV, ms) records as a function of the different repetitions.

highest HR values, while subsequent trot and walk phases yielded higher values compared to their pre-gallop counterparts. When analyzing HR variability (HRV), greater variations were observed at the beginning of the exercise compared to



Figure 1: Heart rate (HR, bpm) and heart rate variability (HRV, ms) records as a function of the different breeds.

Heart rate (HR) is a primary criterion evaluated during veterinary inspections to assess the recovery of horses after a race [1]. In the present study, three Anglo-Arabian horses (3-6 years old) and two Quarter Horses (3-6 years old) participated in three training sessions spaced 7 days apart. The training consisted of Phase I (Walk) for 5 minutes, followed by Phase II (Trot) for another 5 minutes, Phase III (Gallop) for another 5 minutes, Phase IV (Trot after gallop) for 2 minutes, and Phase V (Walk after gallop) for 3 minutes. External sensors (V800, Polar, Bethpage, NY, USA) were attached to the horses' thoraxes with straps, and data were recorded using the Polar Sensor Logger APK software. The gallop phase produced the

Phases IV and V, where response times were significantly shorter than those recorded at the beginning of the training. All exercises showed statistically significant differences ($p < 0.001$) among them (Figure 1), consistent with previous findings by Younes et al. [1]. Exercise repetitions did not indicate signs of cardiac resistance; HR values increased across all exercises with successive repetitions. Regarding HRV, similar trends were observed, although slower cardiac responses (higher HRV values) were apparent during the gallop and subsequent phases in the final repetition. More than three repetitions are necessary to observe improvements in HRV and reductions in HR, as suggested by Janczarek et al. [2] (Figure 2). Quarter Horses exhibited significantly higher HR values during walking and trotting, while displaying lower HR values during the gallop and subsequent phases. Their HRV was higher than that of Anglo-Arabian horses during these exercises (Figure 3), demonstrating an innate ability to adapt to more intense activities—a breed-specific characteristic [3]. In conclusion, Polar equine sensors provide an excellent method for monitoring cardiac function during equine training. At the beginning of the exercise, HR is lower and HRV is higher, indicating low stress levels. Three repetitions do not result in significant cardiac adaptation. Differences in cardiac capacity can be observed between the breeds studied.

[1] Younes, M., et al. (2016). *Frontiers in physiology*, 7, 155.

[2] Janczarek, I., et al. (2013). *Veterinary Science*, 33(10), 794–801.

[3] Castejón, F., et al. (1994). *Journal of Veterinary Medicine Series A*, 41(1–10), 645–652.

Unveiling sleep in horses: monitoring lying behaviour in different housing conditions

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In mammals, sleep is fundamental for physiological and cognitive functions. To avoid sleep deprivation, horses need to lie down daily for an adequate amount of time [1, 2]. However, several factors can affect lying behaviour in horses, including housing conditions and management practices [2]. Precision tools, such as triaxial accelerometers, have been proven to be reliable devices for continuously, non-invasively and accurately monitoring of the horses' lying behaviour [3]. This study aimed to monitor lying behaviour in horses kept in two different housing conditions during the night hours (from 20 to 9:30). The lying behaviour of 10 Lipizzan riding school horses (6 mares and 4 geldings, 14.8 ± 3.7 years old) was continuously monitored for two separate five-day periods in two different housing conditions: Single boxes (horses were kept in single boxes for 24 hours) and Social group (horses were kept in single boxes from 9:30 to 20 and in group paddock during the night). Each horse was monitored twice, once in each condition. The MSR145 accelerometer (MSR Electronics GmbH, Seuzach, Switzerland) was applied to the metacarpal bone of the left front leg (see Figure 1a). A Friedman test was conducted to evaluate the impact of housing conditions on lying behaviour, specifically in terms of daily duration, frequency, and/or bout duration. Although not statistically significant, the mean daily lying duration in the social group was longer than in the single box, at 35.5 ± 27.7 min/day and 29.6 ± 22.2 min/day, respectively (see Figure 1b). No significant differences were found between housing conditions for both frequency and duration of lying bouts (2.4 ± 1.1 bouts of 12.8 ± 9.4 min in social group; 2.1 ± 0.9 bouts of 12.7 ± 5.6 min in single box).



Figure 1. a) Accelerometer placed on the left front leg, metacarpal region; b) Mean daily lying duration over 5 days.

Although not significantly, our results seem to support the hypothesis that larger space, an open view and the presence of conspecifics could encourage lying, as the horse feels more comfortable and safer under such conditions [4]. Furthermore, the triaxial accelerometer as a non-invasive, well-tolerated and cost-effective tool was found useful for monitoring lying, thus offering valuable insights into equine welfare under different housing conditions and detecting sudden changes. However, since the group of subjects involved exhibited a lower mean daily lying duration compared to values reported in the literature [4], further research should aim to enlarge the sample size and consider management conditions different from those outlined in the current study.

- [1] A. Haines, "Sleep deprivation in horses." [Online]. Available: <https://www.veterinary-practice.com/article/sleep-deprivation-in-horses>
- [2] Z. Kelemen, H. Grimm, M. Long, and U. Auer, "Recumbency as an Equine Welfare Indicator in Geriatric Horses and Horses with Chronic Orthopaedic Disease," pp. 1–16, 2021.
- [3] M. Williams and S. Hatto, "Evaluation of accelerometer fitting position, sampling intervals and data editing techniques for measuring equine lying postures when stabled," *Appl Anim Behav Sci*, vol. 266, no. August, p. 106036, 2023, doi: 10.1016/j.applanim.2023.106036.
- [4] L. Greening and S. McBride, "A Review of Equine Sleep: Implications for Equine Welfare," *Front Vet Sci*, vol. 9, no. August, pp. 1–22, 2022, doi: 10.3389/fvets.2022.916737.

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Monitoring Behavior in Heat-Stressed Dairy Buffaloes: General Pattern and Effect of a Nutritional Strategy

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The use of wearable sensors in precision livestock farming is gaining attention to monitor animal behavior and improve farm management. Ear sensors represent a non-invasive tool for tracking key behavioral patterns in livestock including rumination, feeding, resting, and activity time. This study aimed to evaluate different behavioral patterns related to THI levels in dairy buffaloes.

Three groups of 19 dairy buffaloes were formed. Control group (C) received the basal diet, while the two treatment groups (T1 and T2) received the basal diet plus 100 or 200 g/head/day of a nutritional supplement (RuFO, AHV, Italy) to optimize the rumen fermentation and increase nutrient digestibility. The experiment lasted 93 summer days. In the barn, there were no cooling systems. Environmental conditions, such as temperature and humidity, were monitored through three dataloggers (one for each group). Animal behavior was monitored using ear tags (CowManager, Netherlands) which continuously provided information on nutrition, health, and reproduction by measuring eating time, rumination time, rest time, activity time, and high activity time. Data was analyzed using GLM with Group and THI classes (five in total) as fixed factors and animal ID as random and nested within the group. In addition, a regression between THI classes and average group data of each parameter was performed. To prevent misunderstandings, the data from 7 pm to 9 am were discarded, focusing the analysis on behavior during daylight hours (from 10 am to 6 pm). The goal was to assess the response during the hottest hours; therefore, the confounding effect of nighttime-low THI, which could result in behavior not directly linked to THI, was removed.

Results showed that both treated groups spent more time ruminating ($p<0.01$) compared with C group across all THI classes. Also, the activity was different: rest time was higher ($p<0.01$) for the C and T2 groups compared with T1 in all THI classes. Additionally for all groups, as THI increased, eating ($R^2=0.89$, $p<0.05$) and high activity ($R^2=0.90$, $p<0.05$) tended to rise, whereas resting ($R^2=0.83$, $p<0.05$) and active time ($R^2=0.99$, $p<0.05$) tended to decrease. Regarding rumination, an increase was observed up to $THI<79$, after which it declined ($R^2=0.95$, $p<0.05$).

In summary, dairy buffaloes displayed varying behaviors based on environmental conditions, and RuFO also had a beneficial effect on animal welfare, improving especially rumination time, particularly in T1. Wearable sensors are a promising tool for understanding differences between animals and could assist in deepening animal behavior.

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Activity of native Spanish roosters (Castellana Negra and Pita Pinta) in response to hens of different breeds, measured by actigraphy.

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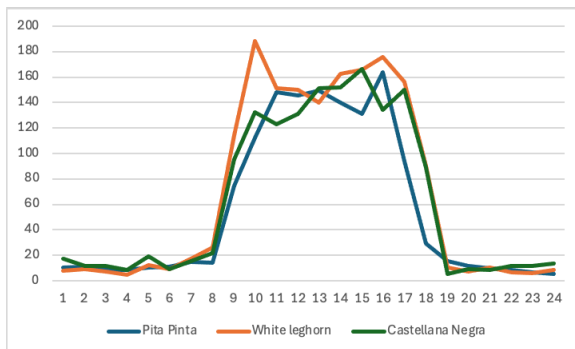


Figure 2: Hourly distribution of hen activity records (MV) by Pita Pinta (PP), White Leghorn (HL) and Castellana Negra (CN) breeds.

Accelerometers mounted in harnesses on laying hens are very useful to measure their locomotor activity [1]. The aim of this study was to record the active behaviour of hens together with roosters in outdoor pens using triaxial accelerometers as movement sensors. A study was carried out in two pens: the first with a rooster of the Castellana Negra (CN) breed (LW: 2.3 kg) and the second with a rooster of the Pita Pinta (PP) breed (LW: 3.5 kg). In the first pen they were with two Castellana Negra (CN) hens and two White Leghorn (HL) hens. In pen 2 they were kept with two Pita Pinta (PP) and two White Leghorn (HL) hens. The animals were housed in an indoor facility (0.75 m²/bird), attached to an outdoor run (10 m²/bird). For seven days they were fitted with a harness equipped with an accelerometer (wGT3X-BT; ActiGraph, FL, USA) (46x33x15 mm; 19 g) to record

activity data, measured by the so-called Magnitude Vector, at 1-second intervals (activity counts/second). Proximity data between the hens and the rooster was estimated with a Bluetooth function between the devices from the RSSI (Received Signal Strength Indicator) values. The data of the magnitude value and the distance between them were analysed with the SPSS statistical package. We found different activity ($p < 0.001$) between the two sexes. Roosters recorded higher activity (102.5 ± 0.65) than females (62.5 ± 0.21). Significant differences were recorded between the breeds used, in males the most active were the CN (118.67 ± 0.84), and among the hens, the HL (66.67 ± 0.34); in both cases the least active were the animals of the PP breed. All the animals performed circadian activities with different activity records between the hours of the day, according to sex (Figure 1) and breed (Figure 2). Regarding the proximity between hens and cocks, there were differences ($p < 0.001$) between each hen and each male, so there seems to be no specific predilection for males or females [2]. There were differences between the breeds studied, the most distant hens were those of the CN breed 2.17 ± 0.004 m and the closest were those of the PP breed 1.75 ± 0.005 m as also described by [3]. In conclusion, actinography allows us to know the behaviour of roosters and hens in freedom. The distances covered are greater for the males than for the females and are different for the three breeds studied.

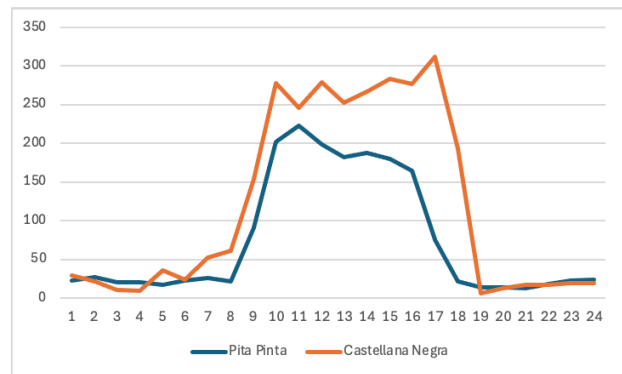


Figure 1: Hourly distribution of activity records (MV) of roosters by Pita Pinta (PP) and Castellana Negra (CN) breeds..

- [1] Abecia, J. A., et al. (2024). Journal of Applied Poultry Research, 33(4).
- [2] Abeyesinghe, S. M., et al. (2013). Applied Animal Behaviour Science, 143(1), 61–66.
- [3] Tiemann, I., and Rehkämper, G. (2008). Brain Research Bulletin, 76(3), 300–303

Precision beekeeping systems as tools for monitoring bees to agroecosystems interactions.

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Precision Beekeeping (PB) employs innovative systems to monitor hive conditions, offering to beekeepers real-time data on internal hive temperature, humidity and sound, hive weight, and bee flight activity with the aim of enhancing colony health and productivity [1-2]. This study, conducted within the AGRITECH project funded by the European Union NextGeneration EU (Grant number J83C22000830005), focuses on applying precision beekeeping systems (PBS)[3] as tool to test the influence of two different farming systems on the health and activity of honey bee colonies as relevant element of ecosystem service provision. Two apiaries (RSA and RSB) located 1650 meters apart were established in May 2024 at an experimental site (Roccarespampani, Monte Romano, Viterbo), each consisting of four *Apis mellifera ligustica* Spin. colonies housed in 10-frames Italian Dadant hives. The RSA site (20 ha) follows an optimized crop rotation designed to enhance ecosystem services, including sorghum, triticale, alfalfa, clover/raygrass mixture, and flowering strips. Conversely, the RSB (20 ha) is managed according a conventional crop rotation, primarily based on sorghum and clover/raygrass mixture. All the beehives were equipped with PBS from Melixa Srl (Trento, Italy) with sensors for internal and external temperature monitoring, hive weight, and bee flight activity. Data were collected at 15-minute intervals from May to December 2024. To contextualize the observed variations in beehive variables, a land use analysis was conducted using ArcGIS Pro 3.2.0 (Esri Inc.), assessing food resource availability within a 2000-meter buffer around each apiary. Land categories included coppice forest, cultivated fields, pastures, and other relevant types, enabling a comparative evaluation of resource distribution. During the study period, the RSA colonies shown a higher ($p<0.05$) internal temperature (17.4 ± 6.0 °C) during the late months of the year (November and December) compared to the RSB colonies (12.5 ± 6.8 °C). In the RSA, colonies exhibited a significant weight increase ($p<0.05$) compared to RSB in the study period (Fig. 1) The greater weight of the RSA colonies, indicated a higher number of bees, larger honey and pollen stores, and the presence of brood until mid-November, confirmed by periodic inspections carried out in the study period. Flight activity was comparable between the two apiaries until August (83 ± 155 and 124 ± 240 flights/15 min for RSA and RSB, respectively). The land use analysis indicated that both sites exhibit a predominance of non-irrigated arable land, covering 60.4% of RSA and 54.2% of RSB, followed by oak forests (27.9% vs. 30.6%). The land use analysis for each category, despite some small differences between them, the Mann-Whitney U test ($p>0.05$) showed no statistically significant difference in the overall land use distribution between the two areas. A long-term study is ongoing to further explore the colonies dynamics and provide deeper insights into the interaction between agricultural practices and honey bees as *proxi* of other potentially relevant pollinators. For such type of studies on honey bee colonies, PB systems are pivotal thanks to the great amount of real-time data they can produce at rather low cost.

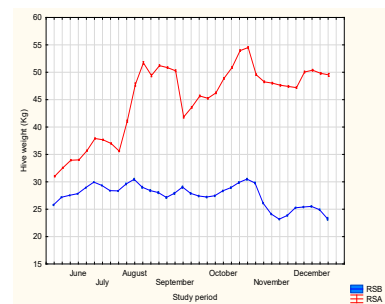


Figure 1. Hive weight (mean \pm SE) variation of the two apiaries under study.

References

- [1] Alleri, M., Amoroso, S., Catania, P., Lo Verde, G., Orlando, S., Ragusa, E., Sinacori, M., Vallone, M., & Vella, A. (2023). Recent developments on precision beekeeping: A systematic literature review. *Journal of Agriculture and Food Research*, 14(June), 100726. <https://doi.org/10.1016/j.jafr.2023.100726>
- [2] Zacepins, A., Kviesis, A., Stalidzans, E., Liepniece, M., & Meitalovs, J. (2016). Remote detection of the swarming of honeybee colonies by single-point temperature monitoring. *Biosystems Engineering*, 148, 76–80. <https://doi.org/10.1016/j.biosystemseng.2016.05.012>
- [3] Danieli, P. P., Addeo, N. F., Lazzari, F., Manganello, F., & Bovera, F. (2023). Precision Beekeeping Systems: State of the Art, Pros and Cons, and Their Application as Tools for Advancing the Beekeeping Sector. *Animals*, 14(1), 70. <https://doi.org/10.3390/ani14010070>.

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Assessment of Plasma Treated Seawater (PTSW) in shellfish depuration systems

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Shellfish can bioaccumulate several foodborne pathogens, especially enteric viruses like human norovirus (HuNoV) posing a significant public health risk since they are often consumed raw or slightly cooked [1]. They are commonly subjected to depuration processes when they came from harvesting area B or C (EU Reg 2019/627) to ensure compliance with microbiological criteria related to bacterial indicators such as *E. coli* and *Salmonella* spp. as mandated by (EC) No 2073/2005 [2,3]. However, the commercial shellfish depuration process has been demonstrated to be ineffective at removing HuNoVs [4]. In order to address this drawback, plasma treated seawater (PTSW) showed promising antiviral activity due to the presence of highly oxidizing environment with reactive oxygen and nitrogen species (RONS) [1]. Therefore, the aim of this study was to evaluate the efficacy of PTSW technology to improve the depuration of experimentally contaminated mussels with F-specific MS2 bacteriophages ($5.08 \pm 0.05 \log_{10}$ PFU/ml) which are commonly employed as HuNoV surrogates.

PTSWs were generated with a Dielectric Barrier Discharge (DBD) properly modified to treat shellfish. An electric field at 9 kHz frequency and 10 kV peak-to-peak voltage was applied to ignite the discharges, modulated in all experiments at a 50% duty cycle. Synthetic air was used as working gas. The different PTSWs were physico-chemically characterized after and before plasma treatments employing commercial reagents. Shellfish were exposed to PTSWs treatments of 40 and 80 minutes. The inactivation of MS2 bacteriophages was quantified using a double-layer plaque assay. Finally, the impact of PTSWs on mussel viability was evaluated checking the opening of their valves and their responsiveness to external stimuli.

After 40 minutes of treatments PTSW reduced the viral titre of MS2 bacteriophages in experimentally contaminated shellfish of $\approx 0.5 \log_{10}$ PFU/ml. However, after 80 min of PTSW, the reduction of MS2 viral titre reached $1.85 \pm 0.01 \log_{10}$ PFU/ml. Moreover, all PTSWs analysed didn't affect mussel viability.

Our results demonstrated that PTSW treatment of 80 minutes was the most promising and reliable treatment to achieve MS2 viral reduction in experimentally contaminated shellfish. However, further studies are needed to fully evaluate the antiviral efficacy of PTSW technology and the role played by the reactive species in the viral inactivation process.

1. Pandiscia, A.; Lorusso, P.; Manfredi, A.; Sánchez, G.; Terio, V.; Randazzo, W. Leveraging Plasma-Activated Seawater for the Control of Human Norovirus and Bacterial Pathogens in Shellfish Depuration. *Foods* 2024, 13, 850, doi:10.3390/foods13060850.
2. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs with EEA Relevance) Text with EEA Relevance.
3. COMMISSION IMPLEMENTING REGULATION (EU) 2019/ 627 - of 15 March 2019 - Laying down Uniform Practical Arrangements for the Performance of Official Controls on Products of Animal Origin Intended for Human Consumption in Accordance with Regulation (EU) 2017/ 625 of the European Parliament and of the Council and Amending Commission Regulation (EC) No 2074 / 2005 as Regards Official Controls;
4. McLeod, C.; Polo, D.; Le Saux, J.; Le Guyader, F.S. Depuration and Relaying: A Review on Potential Removal of Norovirus from Oysters. *Compr Rev Food Sci Food Saf* 2017, 16, 692–706, doi:10.1111/1541-4337.12271.

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Valorisation of Neglected Fish Species through the Dry-Curing Process: Microbiological Assessment of Thinlip Grey Mullet Fillets

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Figure 1 – Logo of the PRIN 2022NX5FL8 project.

Fish dry-curing involves preserving fresh fish in dedicated cabinets to enhance its flavour and texture. Like meat, fish is hung or placed on racks in cabinets with controlled environmental parameters for several days. Initially adopted by a few chefs, this technique is now gaining popularity in restaurants and food companies [1,2]. However, data on the microbiological characteristics and safety of dry-cured fish remain limited [1,2,3]. Dry-curing could also be used to valorise neglected and underutilised fish species (NUS), in order to obtain innovative ready-to-eat (RTE) products more attractive to consumers. As part of the PRIN 2022NX5FL8 project, thinlip grey mullet (*Liza ramada*) was identified as a suitable NUS for creating RTE fish products through dry-curing. The aim of this study was to investigate the microbiological profile of thinlip grey mullet fillets during dry-curing and the subsequent shelf-life of the resulting RTE products.

Frozen thinlip grey mullets were manually filleted, cured with a salt-spice mix, and placed in a dedicated cabinet. The dry-curing process involved a curing phase followed by dripping and several drying steps. The process parameters (time, temperature, relative humidity, ventilation) were monitored and controlled by an integrated software. After dry-curing, the fillets were vacuum-packed and stored at 2°C for 63 days. Microbiological analyses were performed during various dry-curing steps and throughout the shelf-life. The enumerations of mesophilic and psychrophilic bacteria, *Enterobacteriaceae*, *Pseudomonas* spp., lactic acid bacteria, yeasts and moulds, and *Clostridium* spp. were performed. In addition, representative colonies grown on the different culture media were identified using MALDI-TOF MS, and product characteristics (pH and a_w) were monitored.

At the end of the dry-curing process, total mesophilic and psychrophilic bacteria reached approximately 6 log₁₀ CFU/g, while lower levels were observed for the other microbiological indicators. Microbial loads remained relatively stable throughout the entire shelf-life of vacuum-packed fillets, up to day 63. Suspected *Clostridium* colonies and moulds were never detected. MALDI TOF-MS analyses revealed that the microbial community at the end of the shelf-life was dominated by *Brochothrix*, *Psychrobacter*, and *Carnobacterium*.

These preliminary results highlight that dry-curing, conducted under controlled temperature, relative humidity, and ventilation, is a valuable tool for enhancing NUS while maintaining microbiological quality and safety characteristics. Further studies are needed to confirm the potential of this technology in the valorisation of other fish species and for the risk assessment of the derived RTE products.

[1] V. Indio, F. Savini, F. Gardini, F. Barbieri, L. Prandini, Y.T. Mekonnen, F. Tomasello, F. Giacometti, A. Seguino, A. Serraino, A. De Cesare, "Microbiological safety of dry-cured fish from the raw material to the end of processing", *International Journal of Food Microbiology*, 415, 110641, 2024.

[2] F. Panebianco, M. Nobile, G. Pasinetti, D. Pattono, S. Panseri, T. Civera, "Cured or fresh? Between fish maturation trends in restaurants and food safety: the case of dry-aged rainbow trout", *Food Control*, 110612, 2024.

[3] F. Savini, F. Giacometti, F. Tomasello, V. Indio, F. Gardini, F. Barbieri, L. Bardasi, M. Ramini, L. Prandini, Y.T. Mekonnen, S.A. Cuomo, A. De Cesare, A. Serraino, "Impact of fish dry-curing on the behaviour of *Listeria monocytogenes* during the production of ready to eat fishery products", *LWT*, 204, 116381, 2024.

Analytical Measurement of Goat Cheese Aroma: use of Electronic Nose Technology to identify products and to value their quality

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Figure 1 - Objective olfactory assessment with electronic nose

Cheese aroma is a critical parameter for consumers when assessing their quality [1]. The electronic nose equipped with artificial intelligence and ten different sensors enables an objective olfactory assessment for discrimination between multiple samples to identify variety differences, predict freshness and storage time [2].

This study utilized the PEN3 instrument to evaluate differences among four goat cheeses (two from the Syrian Derivative and two from the Potenza goat breed) and their vicinity with the feed (hay and pasture) provided to the animals. In this regard, four primo sale cheese samples were collected from four Italian farms (C. and P. with Syrian Derivative, T. and A. with Potenza goat breed) located in the Basilicata region (Potenza province). Corresponding feed samples (hay and/or pasture) provided to the animals that produced the milk used for the cheese production, were also collected.

Data analysis utilized the pattern recognition method PCA (Principal Component Analysis), employing the score plot model. The results revealed that cheeses from the A. and C. farms exhibited a positive correlation with the corresponding hay (0.977 and 0.919, respectively) and a negative correlation with the pasture (0.326 and 0.315, respectively), index of a diet primarily based on hay, supplied in greater amounts than pasture. Conversely, primo sale cheese from the P. farm showed a nearly equal proportion of hay and pasture in the animal's diet, as demonstrated by an almost completely overlapping positive correlation in the aromatic profile of both (0.985 and 0.986). Finally, also in the T. farm, where feed consisted exclusively of pasture, a positive correlation (0.967) was observed between the final product and the feed. The electronic nose with sensors detecting specific molecular groups (e.g., sulfur-organic, methane-aliphatic, sulfur-chloride, hydrogen compounds, etc.), not only allowed for comparison between the aromatic composition of the raw material and the final product but also for the identification of the cheese with the superior aromatic quality and lower degradation. Probably due to its production technology, A. primo sale emerged as the best-performing cheese, showing significantly lower levels of most "undesirable" molecules (associated with higher degradation) such as sulfuric-organic compounds, broad-methane, broad-alcohol and sulfuric-chloride.

In conclusion, these preliminary experimental data demonstrate that the use of the electronic nose can provide a statistical correlation between raw materials and final products, while also offering objective evaluations of the aromatic qualities of cheese. This data lays the groundwork for expanding the research to include temporal correlations during cheese ripening.

[1] Drake, M. A., Gerard, P. D., Kleinhenz, J. P., & Harper, W. J. (2003). Application of an electronic nose to correlate with descriptive sensory analysis of aged Cheddar cheese. *LWT-Food Science and Technology*, 36(1), 13-20.

[2] Ampuero, S., & Bosset, J. O. (2003). The electronic nose applied to dairy products: a review. *Sensors and Actuators B: Chemical*, 94(1), 1-12.

Application of Next-Generation Sequencing Technologies to Insect-Based Products: Species Authentication and Microbiome Characterisation for Risk Assessment

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The mass rearing of edible insects presenting a viable alternative to traditional livestock, responding to the escalating demand for sustainable animal protein sources [1]. However, despite the anticipated production of 260,000 tons of insect-based products (IBPs) by 2030, consumer acceptance within the European Union (EU) remains low [2]. In accordance with Regulation (EU) No 2015/2283, IBPs are classified as Novel Foods and can be marketed in the EU only after a positive EFSA risk assessment [3]. Currently, five food business operators are authorized to market IBPs made from four edible insect species: *Tenebrio molitor*, *Locusta migratoria*, *Acheta domesticus* and *Alphitobius diaperinus*. IBPs are mainly sold on the EU market through e-commerce [4], which increases the risk of unauthorised or mislabelled IBPs reaching consumers and poses significant challenges for Competent Authorities.

In this study, Next Generation Sequencing technologies (NGS) were applied to the analysis of IBPs sold online in the EU market. In detail, metabarcoding was used for IBPs authentication (identification of insect species used for IBPs production) and for microbiome characterization. Data obtained from this study might contribute to a more targeted risk assessment in this type of Novel Food.

Forty-six processed IBPs were collected from nine European e-commerce platforms (e-CO). For the authentication, a 200 bp region of the *16S rRNA* gene was amplified and sequenced. For the microbiome characterization (performed on 40 of the 46 IBPs) the V3-V4 region of the same gene was used. In both cases, sequencing data were processed using the bioinformatic pipeline DADA2 in R. In the authentication analysis, a mislabelling rate of 33.3% was observed, particularly involving IBPs made of *A. domesticus*. The use of unauthorized species (e.g. *Gryllus locorojo*), the partial replacement of high-value species with lower-value ones. With respect to the microbiome results, *Aeromonadaceae* and *Bacillaceae* were found the most abundant families and *Aeromonas*, *Bacillus*, *Clostridium*, *Staphylococcus* and lactic acid bacteria (LAB) were detected as genera of major interest for their potential impact on food safety. *Aeromonas* spp. was detected in processed IBPs for the first time, highlighting the need for further assessments. Finally, powdered and ready-to-eat IBPs appeared particularly susceptible to contamination by spoilage bacteria genera

The present study supports the risk assessment of poorly characterised novel foods distributed in the EU, confirming that IBPs sold online should be properly monitored.

[1] Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P., "Edible insects: Future prospects for food and feed security", *Food and agriculture organization of the United Nations (FAO)*, vol. 171, 2013.

[2] Mancini, S., Sogari, G., Diaz, S. E., Menozzi, D., Paci, G., & Moruzzo, R. "Exploring the future of edible insects in Europe", *Foods*, vol. 11, pp.455, 2022.

[3] Sogari, G., Amato, M., Palmieri, R., Hadj Saadoun, J., Formici, G., Verneau, F., & Mancini, S., "The future is crawling: Evaluating the potential of insects for food and feed security", *Current Research in Food Science*, vol. 6, 2023.

[4] Pippinato, L., Gasco, L., Di Vita, G., & Mancuso, T., "Current scenario in the European edible-insect industry: A preliminary study", *Journal of Insects as Food and Feed*, Vol. 6, pp. 371–381, 2020.

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Using Cricket Flour (*Acheta domesticus*) as an Additional Source of Protein in the Production of Freshwater Fish Patties

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The global population is expected to exceed 9 billion by 2050, which will raise demand for crops and livestock, particularly animal protein [1]. As a result, alternative protein sources are becoming increasingly popular as customers seek more innovative and environmentally responsible solutions. Edible insects [2] can be used to replace fish or to create hybrid fish products. This study examined the incorporation of cricket (*Acheta domesticus*) flour in freshwater fish patties and its impact on composition, microbiological, and rheological properties. Three different formulations of fish patties were prepared. The control was composed of ground fillets obtained from freshwater fish (*Cyprinus carpio*, L.-carp) (83%), mashed potatoes (10%), egg white (4.6%), water (1.5%), salt (0.6%), and 0.3% chopped fresh parsley. The other two formulations were made by combining 2.5% (2.5CFP) and 5% (5 CFP) of cricket flour (CF); an equivalent amount of fish flesh was substituted. Including CF led to CFP with significantly higher protein levels than the CTR (17.49 %, 19.46 %, 21.20% in CTR, 2.5% CFP and 5% CFP respectively). Regarding the fatty acids profile, lending of CF at two levels modifies the fatty acids composition. SFA increased as the concentration of CF increased (30.77 %, 32.30 %, 37.57% in CTR, 2.5% CFP and 5% CFP respectively) on the contrary MUFA decreased (43.37 %, 42.10 %, 38.75% in CTR, 2.5% CFP and 5% CFP respectively) as the concentration of CF increase while PUFA were different only between the highest 5CFP and CTR (25.86%, 25.60 %, 23.68% in CTR, 2.5 CFP and 5 CFP). The n-6/n-3 ratio was highest in the treated sample especially in 5CF than in the CTR due to a higher concentration of n6 in the CF (1.22, 1.44, 2.23 % in CTR, 2.5 CFP and 5 CFP respectively). No differences in the total viable count of *Enterobacteriaceae* were registered among the sample. No differences in pH, in any of the cooked samples were registered. Regarding rheological properties, CF integration causes a slight effect on the elasticity of the cooked product and the gumminess and cohesiveness of the raw product. The integration of CF progressively altered the colour parameters, such as lightness, red index, and yellow index, leading to products with a visibly darker, brownish colour, which could be a critical factor for consumer sensory acceptance [3].



Figure 1 Fish patties, CTR (control), 2.5 CFP (2,5 %cricket flour added to patties, 5CFP (5% cricket flour added to patties)

[1] Cavalheiro, C.P.; Ruiz-Capillas, C.; Herrero, A.M.; Pintado, T.; Cruz, T.M.P.; Silva, M.C.A. *Innov. Food Sci. Emerg. Technol.* 2022, 83, 103245.

[2] Melgar-Lallane, G.; Hernández-Álvarez, A.J.; Salinas-Castro, A. *Compr. Rev. Food Sci. Food Saf.* 2019, 18, 1166–1191.

[3] Pasqualin Cavalheiro, C., Ruiz-Capillas, C., Herrero, A. M., Pintado, T., Avelar de Sousa, C. C., Sant'Ana Falcão Leite, J., & Costa Alves da Silva, M. (2024). *Foods*, 13(2), 286.

Determination of iodine content in dairy food matrices

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Iodine deficiency is widespread globally and is the leading preventable cause of brain damage. Iodine deficiency in pregnant women and children can lead to cognitive impairments, miscarriages, and infant mortality, while adults can cause hypothyroidism and goiter. In this context, dairy foods represent an important dietary iodine source, providing about half of the iodine recommended daily intake in children living in Norway and United Kingdom [1]. Many studies have been focused on the characterization of milk iodine content, but few information is available on other dairy matrices such as ricotta cheese and yogurt, thus limiting knowledge on their contribute in meeting the overall daily iodine requirement (150 µg/day, for an average adult). Based on this background, this study aimed to i) characterize milk, ricotta cheese and yogurt for their iodine content, and to ii) investigate differences among milk, ricotta cheese and yogurt iodine content.

Whole pasteurized milk (WM; n = 11), partially skimmed pasteurized milk (PM; n = 19), skimmed pasteurized milk (SM; n = 7), ricotta cheese (RC; n = 26), whole yogurt (WY; n = 13) and low fat yogurt (LY; n = 15) were purchased in local stores, for a total of 91 samples. Samples underwent a preparation protocol and were analysed through inductively coupled plasma optical emission spectrometry for iodine quantification [2] (Fig. 1). Data were analysed through a generalized linear model including iodine concentration as dependent variable and sample type as fixed effect (6 classes: WM, PM, SM, RC, WY and LY). Differences between least squares means were assessed using Bonferroni's post hoc multiple comparison ($P < 0.05$).

Among studied matrices, LY and RC exhibited the greatest average iodine concentration (289.33 and 260.00 µg/kg, respectively), while WM the lowest (190.91 µg/kg). Intermediate concentrations were observed for PM (234.47 µg/kg), SM (207.86 µg/kg) and WY (258.46 µg/kg). In the light of these results, a typical serving of milk (200 g) would provide from 25.45 to 31.26 % of the average daily iodine requirement, a serving of ricotta (40 g) 10.40 %, and a yogurt jar (125 g) from 21.54 to 24.11 %. Overall, this study confirmed the importance of milk and dairy products as a primary iodine sources in the daily diet.

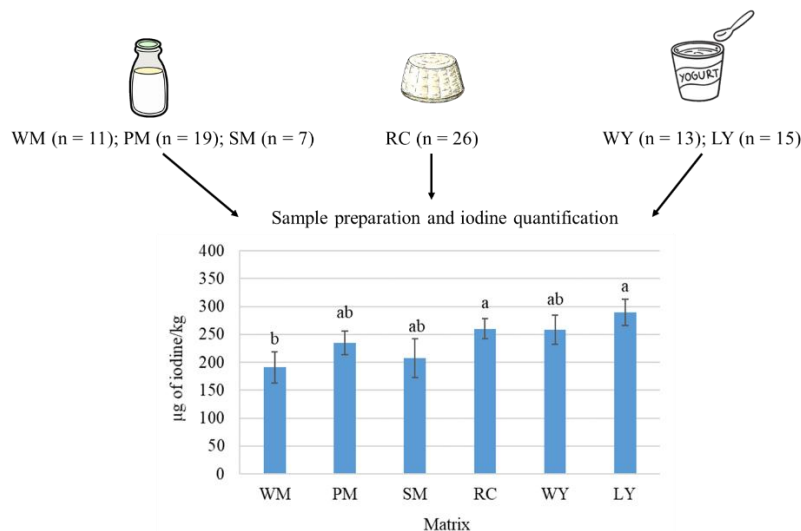


Fig 1. Experimental design and least square means (SE) for iodine concentration in whole pasteurized milk (WM), partially skimmed pasteurized milk (PM), skimmed pasteurized milk (SM), ricotta cheese (RC), whole yogurt (WY) and low fat yogurt (LY). Different letters refer to $P < 0.05$.

[1] S. C. Bath, J. Verkaik-Kloosterman, M. Sabatier, S. Ter Borg, A. Eilander, K. Hora, B. Aksoy, N. Hristozova, L. van Lieshout, H. Tanju Besler, and J. H. Lazarus, "A systematic review of iodine intake in children, adults, and pregnant women in Europe-comparison against dietary recommendations and evaluation of dietary iodine sources". *Nutr. Rev.* vol. 80, pp. 2154–2177, 2022.

[2] G. Niero, M. Franzoi, V. Vigolo, M. Penasa, M. Cassandro, C. Boselli, G. Giangolini, and M. De Marchi, "Validation of a gold standard method for iodine quantification in raw and processed milk, and its variation in different dairy species." *J. Dairy Sci.* vol. 102, pp. 4808-4815, 2019.

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Measurement of antioxidant properties of Lactic Acid Bacteria (LABs) isolated from Buffalo Milk

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Recently, interest in the potential of Lactic Acid Bacteria (LABs) strains as natural additives has increased due to their antioxidative and antimicrobial activity, which can delay food oxidation and extend the shelf life of foods [1]. Buffalo milk represents a valuable resource of LABs strains and functional compounds that can be used for functional food development [2]. Several studies [3, 4] have studied the antioxidant activity of buffalo milk, attributing it to its proteins and peptides recognized as health benefits. In this regard, the antioxidative properties of buffalo milk could be linked not only to its intrinsic bioactive compounds but also to the metabolic activities of naturally occurring LABs strains, which produce bioactive peptides and antioxidants during fermentation [5]. Despite the recognized antioxidative potential of buffalo milk, the specific contribution of LABs to these properties remains largely unexplored. Therefore, in the present study, the potential antioxidant activity of selected LABs strains isolated from buffalo milk, was measured for their *in vitro* scavenging activity against hydroxyl and DPPH free radicals, their resistance to hydrogen peroxide, total antioxidant capacity (TAC) and Ferric reducing antioxidant power (FRAP).

The results indicated strain-specific antioxidative responses. LABs strains from buffalo milk displayed varying levels of resistance to oxidative stress induced by hydrogen peroxide, with certain strains maintaining survival rates above 80% at 0.7 mM H₂O₂. According to Li et al., 2012 [6], increased H₂O₂ concentration correlates with decreased survival rates. The hydroxyl radical is the most reactive species involved in lipid peroxidation in food products [7]. The cell-free supernatants (CFS) of all strains demonstrated stronger scavenging hydroxyl radical activity compared to intracellular extracts (CFSE) suggesting that the selected LABs strains can produce secondary metabolites with significant antioxidant activity. The scavenging effect on DPPH free radical for intact cells (IC), CFS and CFSE showed that the IC of all strains demonstrated a higher percentage of DPPH radical scavenging compared to CFS and CFSE. This result suggests that the cell-surface compounds of LABs strains [6], play a significant role in antioxidant activity, as also reported by Shi et al., 2019 [8]. Notably, CFS from strains such as *Lactocaseibacillus rhamnosus* and *Lactiplantibacillus plantarum* demonstrated superior TAC and FRAP values, highlighting their ability to chelate Cu²⁺ and Fe²⁺ ions. These findings underscore the significance of LABs in developing functional foods, providing a natural alternative to synthetic preservatives to reduce the application of synthetic antioxidants in food production.

- [1] Wang, Y., Wu, Y., Wang, Y., Xu, H., Mei, X., Yu, D., Wang, Y., & Li, W. (2017). Antioxidant properties of probiotic bacteria. *Nutrients*, 9(5).
- [2] Garau, V., Manis, C., Scano, P., & Caboni, P. (2021). Compositional Characteristics of Mediterranean Buffalo Milk and Whey. *Dairy*, 2(3), 469–488.
- [3] D’Onofrio, N., Balestrieri, A., Neglia, G., Monaco, A., Tatullo, M., Casale, R., Limone, A., Balestrieri, M. L., & Campanile, G. (2019). Antioxidant and Anti-Inflammatory Activities of Buffalo Milk β -Valerobetaine. *Journal of Agricultural and Food Chemistry*, 67(6), 1702–1710.
- [4] Salzano, A., Neglia, G., D’Onofrio, N., Balestrieri, M. L., Limone, A., Cotticelli, A., Marrone, R., Anastasio, A., D’Occhio, M. J., & Campanile, G. (2021). Green feed increases antioxidant and antineoplastic activity of buffalo milk: A globally significant livestock. *Food Chemistry*, 344(November 2020), 128669.
- [5] Hashemi, S. M. B., Jafarpour, D., & Jouki, M. (2021). Improving bioactive properties of peach juice using Lactobacillus strains fermentation: Antagonistic and anti-adhesion effects, anti-inflammatory and antioxidant properties, and Maillard reaction inhibition. *Food Chemistry*, 365(June), 130501.
- [6] Li, S., Zhao, Y., Zhang, L., Zhang, X., Huang, L., Li, D., Niu, C., Yang, Z., & Wang, Q. (2012). Antioxidant activity of Lactobacillus plantarum strains isolated from traditional Chinese fermented foods. *Food Chemistry*, 135(3), 1914–1919.
- [7] Choe, E., & Min, D. B. (2005). Chemistry and Reactions of Reactive Oxygen Species in Foods. *Journal of Food Science*, 70(9), 28–36.
- [8] Shi, Y., Cui, X., Gu, S., Yan, X., Li, R., Xia, S., Chen, H., & Ge, J. (2019). Antioxidative and Probiotic Activities of Lactic Acid Bacteria Isolated from Traditional Artisanal Milk Cheese from Northeast China. *Probiotics and Antimicrobial Proteins*, 11(4), 1086–1099.

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Controlling *E. coli* STEC in raw milk cheese: the role of curd testing

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Raw milk products are a major source of foodborne infections in humans, primarily due to transmissible pathogens such as Shiga toxin-producing *Escherichia coli* (STEC). Regarding its prevalence, in 2023 in Europe 1.9% of milk and milk products were reported as positive for *E. coli* STEC [1].

As cheese production batches can vary in size, and the distribution of pathogens both within a batch and within an individual cheese wheel is uneven, the curd stage is likely the most homogeneous in cheese production before the formation of individual wheels. Moreover, Miszczycha et al demonstrated in a challenge test that the pathogen concentration can increase over 4 log units in the first 24 hours of cheese production [2].

European Regulation (EC) 2073/2005 on microbiological criteria [3] recommends sampling for the detection of *Escherichia coli* in cheeses at the stage where the highest concentration is expected, which is in the curd. Therefore, we proposed curd sampling to ensure the safety of raw milk products. We have selected thirteen dairy producers located in the Autonomous Province of Trento, which produce various types of cheese, including washed-rind cheeses, semi-cooked cheeses, semi-fresh cheeses, goat cheeses, grating cheeses, and cheeses made from thermized milk.

The producers were also classified according to their place of origin: alpine dairies or cheese factories. We analysed 4,250 curd samples, with 168 samples (3.9%) testing positive for *E. coli* STEC. A total of 772 samples tested presumptive positive; these samples were further tested for serogroup identification and the presence of stx1 and stx2 toxins, and the eae factor.

Regarding the distribution of positive curd samples, there was an initial peak in STEC positivity due to two cheese factories with repeated positive results; the prevalence decreased after consultancy interventions were carried out in dairy farms that supplied milk to those cheese factories. Furthermore, there was another peak correlated with the summer months; this finding could be explained by the normal behaviour of the microorganism, which tends to increase with proper temperatures and changes of farming systems (i.e. alpine dairies).

In conclusion, the analysis of curds allowed us to estimate the prevalence of *E. coli* STEC and prevent non-compliant batches from reaching the market. Moreover, a minimally invasive sampling ensures a fast and practical result.

Cheese wheels produced by a positive or presumptively positive curd were regularly monitored to assess the persistence of *E. coli* STEC during the aging process.

[1] EFSA and ECDC (European Food Safety Authority and European Centre for Disease Prevention and Control), (2024). The European Union One Health 2023 Zoonoses report. EFSA Journal, 22(12), e9106. <https://doi.org/10.2903/j.efsa.2024.9106>

[2] Miszczycha Stéphane D., Perrin F., Ganet S., Jamet E., Tenenhaus-Aziza F., Montel M., Thevenot-Sergentet D. (2013) Behavior of Different Shiga Toxin-Producing *Escherichia coli* Serotypes in Various Experimentally Contaminated Raw-Milk Cheeses. American Society for Microbiology

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

A Smart Harness to Detect Attacks from Predators in Herds

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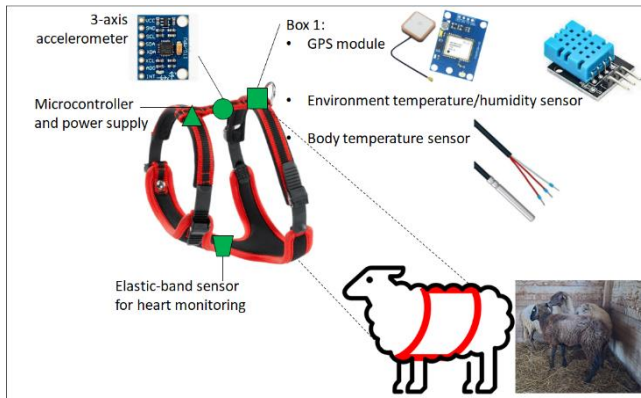


Figure 1 – Sketch of the smart harness implemented to detect attacks from predators in herds.

3-axis accelerometer is used to determine whether the sheep is lying down, standing, or moving, as each behavior results in distinct acceleration patterns. A classification algorithm then analyzes the average acceleration within a specific time window to estimate the animal current state. Furthermore, knowing the animal's heart rate is crucial to identify any rapid increases possibly caused by a predator attack. This is investigated by using a unique elastic-band sensor composed by textile-fabric electrodes, suitable to be applied for prolonged time due to a reliable contact between electrode and animal skin. Lastly, a GPS module measures the animal location with an accuracy adequate for a prompt intervention by the shepherd. Besides, microclimate monitoring in the sheep surrounding is interesting to understand its habits during the day and the conditions it prefers to live. To this aim, the harness is also equipped with a temperature and humidity sensor, along with an additional Pt-100 thermo-resistance located near the animal skin. This enables the measurement of surface temperature, which may differ from that in the surrounding environment, by allowing the detection of temperature increases, which could be caused by factors such as running or prolonged exposure to sunlight. A Proof-of-Concept of the smart harness was implemented with commercial hardware. An experimental characterization with some Massese breed, was conducted at the CiRAA – Agro-Environmental Research Center "Enrico Avanzi" of the University of Pisa. Data acquisition was managed through a microSD. All sensors correctly recorded data with a time sampling of 10 ms for some hours. The offline data-processing demonstrated the good functionality of the system and the potentiality for the application on a large-scale scenario.

[1] <https://europa.eu/eurobarometer/surveys/detail/2996>.

[2] A. Gazzola, C. Capitani, L. Mattioli, M. Apollonio, "Livestock damage and wolf presence", *Jour. Zool.*, vol. 274, pp. 261-269, 2008.

[3] M. Petridou, V. Kati, "Are Wolves the Real Problem? Challenges Faced by Livestock Farmers Living Alongside Wolves in Northwestern Greece", *Sustainability*, vol. 17, n. 3, p. 1083, 2025.

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Evaluating Welfare and Stress in two Slow-Growing Local Poultry Breeds Versus a Commercial Hybrids in a Free-Range Farming System

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Slow-growing local poultry breeds have gained increasing attention for their potential to enhance animal welfare and support biodiversity conservation. Unlike fast-growing commercial hybrids, these breeds exhibit physiological resilience and behavioral adaptability, making them well-suited for extensive farming systems. This study examines key welfare indicators, including hematological parameters, body condition, and behavioral patterns, in two local breeds, Bionda Piemontese (BP) and Robusta Maculata (RM), against the commercial hybrid Ross 308, raised in a free-range system.

Data collection was done at 42 and 84 days of age. At three weeks, birds were grouped by genotype based on average weight, with three replicate per breed (9 pens total). Each pen measured 1.5×1.5 meters indoors and 4×1.7 meters outdoors and housed six birds. A range of physiological and behavioral parameters was assessed to provide an evaluation of welfare conditions. Hematological analysis included the quantification of traditional blood parameters alongside the heterophil-to-lymphocyte (H/L) ratio. Body condition was evaluated through a standardized scoring system for plumage integrity and leg health [1]. Behavioral patterns were analyzed through recorded video sessions taken at two-hour intervals, with ten-minute recordings repeated five times per session (total of 50 minutes per replicate). The primary behavioral categories examined included locomotion, feeding, resting, and social interactions. In addition to these assessments, corticosterone concentrations in both blood and feces were measured to evaluate chronic stress responses.

Results indicated that BP and RM exhibited comparable corticosterone levels, whereas Ross 308 displayed significantly higher concentrations at both 42 and 84 days of age, confirming an elevated physiological stress state. This trend was further supported by hematological findings, where Ross 308 had a higher H/L ratio, indicative of increased systemic stress. Body condition analysis revealed additional welfare discrepancies between the genotypes. Ross 308 exhibited poorer plumage quality, compromised leg health compared to the slow-growing breeds. Behavioral observations showed that Ross 308 spent significantly more time resting, whereas BP and RM displayed increased locomotor activity and more frequent foraging behavior, indicative of improved welfare and greater environmental engagement.

These findings provide evidence that slow-growing local breeds, demonstrate superior welfare outcomes in free-range systems compared to commercial hybrids. The results emphasize the need for genetic selection strategies that prioritize animal welfare, aligning with the principles of sustainable poultry production. Furthermore, this study highlights the critical role of local breeds in fostering biodiversity conservation and promoting resilient farming systems, reinforcing their value as a sustainable alternative to intensive poultry production.

1. Tauson, R. *et al.* Applied Scoring of Integument and Health in Laying Hens. *Anim. Sci. Pap. Rep.* **23**, 153–159 (2006).

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Evaluation of Milk Ejection Physiology in Italian Mediterranean buffaloes using Lactocorder

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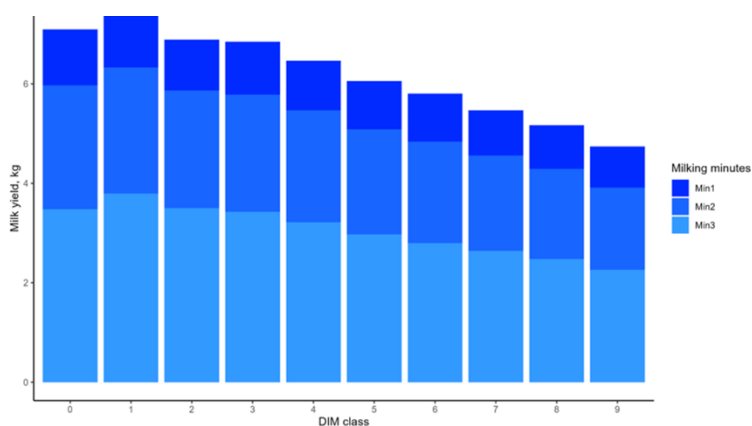
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Figure 1 - First 3 minutes milking related to DIM class.



Buffalo breeding represents a significant economic resource in Italy, particularly in the southern regions. Currently, milking procedures and parameters for buffaloes are largely adapted from dairy cows, despite notable anatomical and physiological differences between the two species [1-2]. This study aimed to enhance the knowledge about milk ejection characteristics in Italian Mediterranean buffaloes (IMB), laying the groundwork for optimizing milking practices. Among the most suitable and specialized devices for evaluating milk flow dynamics, the Lactocorder® measuring device has been considered the gold standard and is one of the most widely employed [3-4]. The study was carried out on four buffalo farms in southern Italy, in-

volving a total of 829 lactating buffaloes. Data collection was performed monthly, in correspondence with the official Performance Recording activity of the Italian Breeders Association. Milking flow data and lactation-related were recorded using ten Lactocorder®. The following parameters were recorded: highest milk flow (HMF), persistence of highest milk flow (HMG), total milking time (tMGG), milk yield in the first three minutes of milking, and over-milking. Data analysis was carried out through a mixed model based on days in milk (DIM) classes (10 DIM, from 0 to 9, each of 30 days) and per parity classes (5 classes: first, second, third, fourth, and fifth lactation).

The highest HMF was observed in DIM class 1 (2.02 kg/min), while the lowest HMF values were recorded in class DIM 9. The highest mean HMF was found in IMB during their second lactation (1.90 kg/min). Similarly, the highest average HMG was recorded in DIM class 1 (1.86 kg/min) with the highest mean HMG also observed in the second lactation (1.74 kg/min). The tMGG showed a decreasing trend as DIM progressed with the longest tMGG occurring in DIM class 1 (7.93 minutes) and in the fifth lactation. DIM class 1 IMB produced the highest milk yield during the second (2.56 kg/min) and third minutes (3.86 kg/min) of milking (Figure 1). The highest average value of overmilking was also recorded in the DIM 1 class.

In conclusion, the findings suggest that IMB exhibits optimal milking performance from the second lactation onward, particularly after 30 days in milk. Further studies are needed to deepen milk ejection physiology and refine milking practices specific to this species.

[1] M. Caria, L. Murgia, and A. Pazzona, "Effects of the working vacuum level on mechanical milking of buffalo", *J. Dairy Sci.*, vol. 94, pp. 1755–1761, 2011.

[2] R. Matera, L. Pacarella, A. Cotticelli, G. Conte, A. Tondo, G. Campanile and G. Neglia, "Milk characteristics and milking efficiency in Italian Mediterranean buffalo," *Ital. J. Anim. Sci.*, vol. 22, pp. 1110–1119, 2023.

[3] M. Wieland and A. Sipka, "Comparison of 2 Types of Milk Flow Meters for Detecting Bimodality in Dairy Cows", *J. Dairy Sci.*, vol. 106, pp. 1078–1088, 2023.

[4] T. Bobić, P. Mijić, V. Gantner, G. Bunevski, and M. Gregić, "Milkability Evaluation of Jersey Dairy Cows by Lactocorder", *Maced. Vet. Rev.*, vol. 43, pp. 5–12, 2020.

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Electroencephalographic analysis of stunning effectiveness in donkeys at slaughter

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Equids slaughtered for human consumption are normally stunned with penetrating captive bolt (PCB) before the act of exsanguination to produce an irreversible state of unconsciousness and ensure welfare. This topic is much studied for the most commonly slaughtered species, while literature is scant on Equidae [1].

While in a commercial setting behavioural signs and reflexes are often used for assessing consciousness during and after stunning, electroencephalogram (EEG) has been shown to provide a more direct indication of brain functional activity and it is considered a more reliable indicator of undoubted unconsciousness [2].

This pilot study aimed to evaluate the EEG of donkeys during stunning by pneumatic PCB and compare it with behavioural signs to assess stunning effectiveness. The study was conducted in a commercial abattoir in Mexico in April 2024. The pressure of the airline could not be recorded as no in-line regulators were present. Tank pressure was 827 kPa. EEG recordings (ADInstruments Ltd) and behavioural signs [3] of 13 donkeys were recorded before, during and after stunning. Donkeys were restrained, EEG recording electrodes placed and baseline EEG recordings conducted (30 seconds).

Afterwards, donkeys were shot by PCB. EEG recordings continued for 60 seconds, with brainstem and behavioural reflexes assessed during and after EEG recordings. Recordings were then analysed offline and categorised into normal-like EEG, movement artefacts, transitional EEG, high amplitude low frequency EEG and isoelectric EEG based on morphology. In addition, waveforms were analysed with fast fourier transformation to allow the assessment of changes in the EEG power spectrum [2].

Forty-six percent (6/13) of donkeys had normal-like EEG after stunning (1 to 9 seconds), indicating a period of potential incomplete concussion. However, in all animals this period was momentary and changed to either transitional EEG or isoelectric waveforms. The periods of normal-like EEG activity were also characterised by increased theta, alpha and beta activity in the EEG power spectrum. Four of these donkeys (67%, 4/6) showed signs of potential incomplete concussion after stunning including rhythmic breathing, gasping, palpebral reflex and muscle spasms.

These results highlight concerns potentially associated with the variability of the airline pressure and maintenance of the PCB. In conclusion, EEG, especially if used in a research setting, could substantially help the understanding of the risks of a potential return of consciousness after stunning. This could assist with the refinement of ideal airline pressure, shot position and validation of behavioural signs that better evaluate consciousness in donkeys.

[1] K. A. Fletcher, G. Limon, L. J. Whatford, A. Grist, T.G. Knowles, and T. J. Gibson, "A systematic review of equid welfare at slaughter" *Livestock Science*, vol. 263, 104988, 2022.

[2] T. J. Gibson, S. E. O. Oliveira, F. A. Dalla Costa, and N. G. Gregory, "Electroencephalographic assessment of pneumatically powered penetrating and non-penetrating captive-bolt stunning of bulls" *Meat Science*, vol. 151, pp. 54-59, 2019.

[3] T. J. Gibson, E. M. Bedford, N. M. Chancellor, and G. Limon, "Pathophysiology of free-bullet slaughter of horses and ponies" *Meat science*, vol. 108, pp. 120-124, 2015.

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Weaning reduces body temperature and heart rate while increasing heart rate variability in ewes

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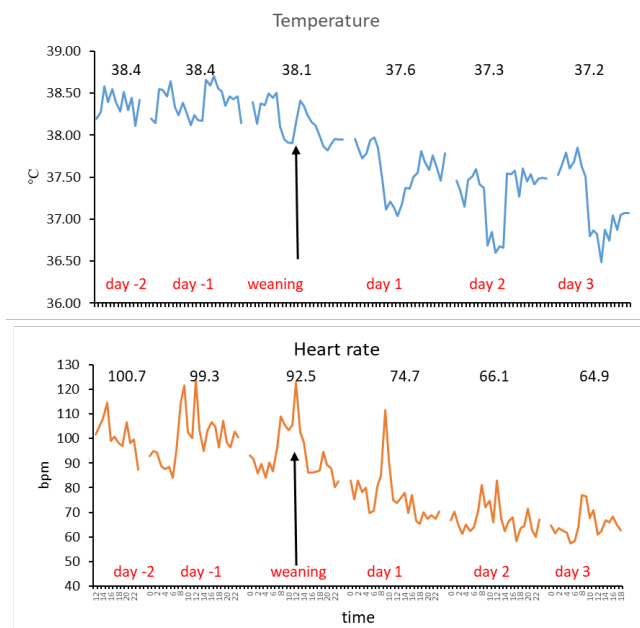


Figure 1 – Mean body temperature and heart rate of ewes before and after weaning.

Weaning elicits strong responses in both ewes and lambs, often adversely affecting their physiology and health, making it a significant welfare concern. However, limited and sometimes contradictory information is available regarding the physiological responses of dams during weaning [1]. The aim of this study was to determine physiological changes in the ewes after weaning. Four days before weaning (d0), 11 lactating ewes received a surgically implanted subcutaneous temperature (T) and heart rate (HR) bio-logger (DST micro-HRT, Star Oddi, Iceland) (8.3×25.4 mm, 3.3 g), programmed to record data every 15 min from d-2 to d3. HR variability (HRV) parameters were calculated based on the raw ECG data recorded between 17:00 and 19:00 h every 5 min, the SDNN (standard deviation of normal to normal R-R inter-vals) and the RMSSD (root mean square of successive differences between normal heartbeats). Sampling was at 200 Hz. Four days after weaning the sensors were removed from the animals and data records downloaded. The day of weaning, ewes and lambs were separated and housed in different barns at 11:30 h. During lactation, ewes were fed a concentrate ration (1 kg of pellets), offered at 0800 h, 1 kg alfalfa hay and barley straw ad libitum; after weaning ewes were fed

0.5 kg barley straw, only. Values before and after weaning were compared by anova; for post hoc analyses among days, the Tukey's multiple comparisons test was used. Body T and HR were similar the two days before weaning, but both variables experienced a gradual decline ($P<0.001$), starting from weaning (Figure 1). On the other hand, HRV parameters (SDNN and RMSSD) presented a significant increase ($P<0.001$) after weaning (Figure 2).

Lactating ewes, due to metabolic work, produce a greater amount of heat than non-lactating ewes, so that the lower T showed after weaning could be a reflect of the interruption of milk production. A reduction of HR has been reported after dry-off in cows, which is probably an effect of the reduced nutrient intake, because a decrease in rumen fill results in a reflex slowing of the heart rate, predominantly because of an increase in parasympathetic tone [2]. This fact could also be responsible of the higher HRV observed in our ewes after weaning, although stress due to the separation of their lambs may also be a factor increasing HRV.

In conclusion, subcutaneous bio-loggers have revealed changes in the physiological parameters of sheep after weaning.

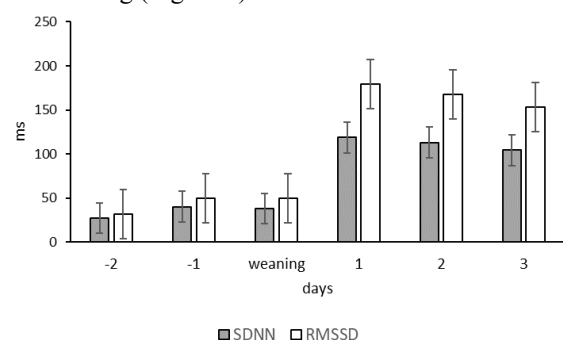


Figure 2 – Mean SDNN and RMSSD of ewes before and after weaning.

[1] A. Freitas-de-Melo, *Front. Anim. Sci.*, vol. 3, Article 823188, 2022.

[2] M. O. Odensten, *J. Dairy Sci.* 90:898–907, 2007.

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Measuring activity and space use overlap between domestic and wild ungulates in a silvo-pastoral system using camera traps

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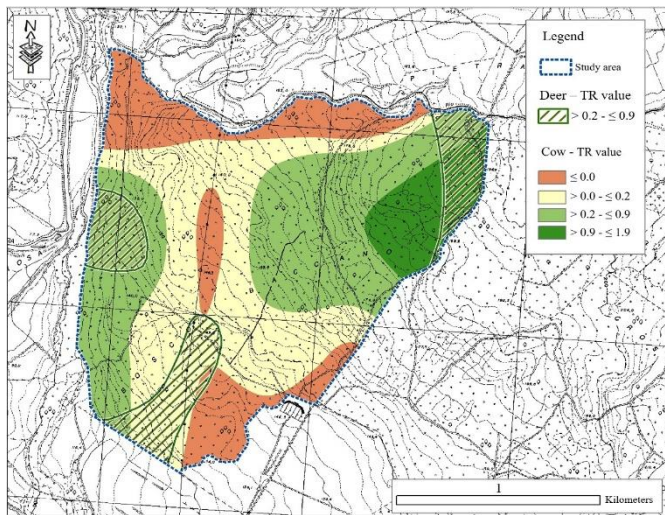


Figure 1- Spatial distribution of Maremmana cattle and red deer in the study area, modelled using Spline analysis. TR=trapping rate.

Understanding animal behaviour and spatial distribution in extensive farming systems is crucial for assessing livestock-wildlife interactions, particularly between sympatric species like cattle (*Bos taurus*) and red deer (*Cervus elaphus*). Thus, this study aimed to measure the extent of temporal and spatial overlap of these two species during winter (January) to understand whether grazing pressure is additive or complementary. The research was conducted in a 2330-hectares farm in Lazio region, central Italy, comprising 565 hectares of pasture and 1765 hectares of woodland, hosting 430 Maremmana cattle and wild species, including the red deer. In five silvo-pastoral sections of the study area, seventeen camera traps were deployed using a systematic sampling approach. Activity rates were analysed with the “activity” package in R [1], while the overlap coefficient was obtained using the “overlap” package [2]. Trapping rate (TR) was calculated as the number of captures per unit

of time. Finally, spatial use was modelled based on TR using Spline analysis in ArcGIS Pro 3.2.0 (Esri Inc.). Cattle exhibited an irregular activity pattern throughout the day, with low activity during the early morning (12-6 a.m.), a moderate peak around 12 p.m., and the highest peak in the late afternoon (6-7 p.m.) followed by a gradual decline. A similar irregularity was observed for red deer, with moderate activity around 12 p.m. and a pronounced peak in the late afternoon (6-7 p.m.). However, compared to cattle, red deer showed a more fragmented activity pattern throughout the day, with multiple smaller peaks. The overlap analysis revealed that cattle and red deer shared 74% of their activity periods, indicating a significant temporal coexistence, but the spatial distribution analysis showed an inverse relationship between cow and deer TR, with deer being more frequently detected in areas of low cow activity (10.82% in TR >0 ≤0.2). As cow TR increased, deer detections decreased, particularly in higher TR categories. Notably, there is a zone (8.88%) where neither species was detected, suggesting an area uninhabited by both. These results confirm that deer and cattle exhibit distinct habitat utilisation patterns, influenced by species-specific foraging behaviours, interspecific competition, and potential avoidance mechanisms [3,4].

This finding highlighted the importance of measuring livestock-wildlife interactions, particularly to assess potential competition for forage resources and possible local depletion of plant biodiversity. The use of monitoring systems capable of continuously measuring spatiotemporal variations, such as camera traps, can inform management strategies for both wildlife and livestock, promoting increased sustainability.

- [1] J. M. Rowcliffe, R. Kays, B. Kranstauber, C. Carbone, P.A. Jansen, “Quantifying levels of animal activity using camera trap data”, *Methods Ecol. Evol.*, vol. 5, pp. 1170-1179, 2014.
- [2] M.S. Ridout, M. Linkie, “Estimating overlap of daily activity patterns from camera trap data”, *J. Agric. Biol. Environ. Stat.*, vol. 14, pp. 322-337, 2009.
- [3] E.R. Loft, J.W. Menke, J.G. Kie, “Habitat shifts by mule deer: The influence of cattle grazing”, *J. Wildl. Manag.*, vol 55, pp.16-26, 1991.
- [4] J.J. Yeo, J.M. Peek, W.T. Wittinger, C.T. Kvale, “Influence of rest-rotation cattle grazing on mule deer and elk habitat use in east-central Idaho”, *J. Range Manag.*, vol. 46, pp. 245-250, 1993.

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An IoT-Driven Smart System for Rams' Physiological status and Activity Monitoring

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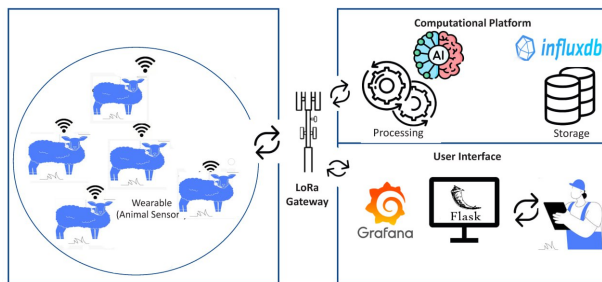


Figure 1: Architecture of An IoT-Driven Smart Monitoring and Analysis System

continuously collects a range of physiological, environmental and motion-related data to provide a comprehensive view of the animal's activity, also allowing early disease detection.

The system collects various data streams, including heart and respiratory rate, body temperature, while ambient temperature and humidity provide insights into environmental conditions affecting the sheep's comfort. Geolocation data (latitude and longitude) is recorded, allowing farmers to track the movement and grazing patterns of individual animals, optimizing pasture management.

An integrated accelerometer captures motion parameters—yaw, pitch, and roll—essential for analyzing activity levels, posture, abnormal behavior's signs such as prolonged inactivity or unusual movement[1], [2]. These motion patterns, combined with physiological indicators, may be valuable to monitor the rams' libido and reproductive activity during breeding season. The continuous data collection facilitates long-term trend analysis, refining predictive models for welfare monitoring.

The collected data are stored in InfluxDB, a time-series database optimized for continuous sensor inputs. Real-time visualization is enabled through Grafana dashboards, allowing farmers and veterinarians to monitor trends and detect irregularities via a React-based user interface. The application incorporates AI-based analytics to classify and cluster ram states such as, resting, running, and other activities [3]. By analyzing historical data, the system identifies activity patterns and distinct states, crucial for monitoring physiological and reproductive status.

Fully containerized with Docker, the system ensures scalability and ease of deployment in diverse farming environments. This IoT-enabled, AI-driven system enhances sheep farming by providing continuous, data-driven monitoring of activity and physiological status. Through real-time tracking, anomaly detection, and cloud-based accessibility, it reduces manual inspections, improves early disease detection, and enhances livestock management. The research highlights the feasibility of smart farming technologies in extensive agricultural systems, offering scalable model for future livestock monitoring solutions.

- [1] N. Kleanthous, A. J. Hussain, W. Khan, J. Sneddon, A. Al-Shamma'a, and P. Liatsis, "A survey of machine learning approaches in animal behaviour," *Neurocomputing*, vol. 491, pp. 442–463, 2022.
- [2] L. Nóbrega, P. Gonçalves, M. Antunes, and D. Corujo, "Assessing sheep behavior through low-power microcontrollers in smart agriculture scenarios," *Computers and Electronics in Agriculture*, vol. 172, p. 105338, 2020.
- [3] L. Wang, R. Arablouei, F. A. Alvarenga, and G. J. Bishop-Hurley, "Classifying animal behavior from accelerometry data via recurrent neural networks," *Computers in Biology and Medicine*, vol. 122, p. 103807, 2020.

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Measurements of diet-related colour changes in the livery and fillets of Gilthead seabream: focus on quality characteristics in the InsectFish project

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Figure 1 – Colour analysis of the fish fillet

The aquaculture sector has seen significant growth in recent years. In 2022 global aquaculture production reached 130.9 million tonnes, valued at USD 312.8 billion and corresponding to 59% of global fisheries and aquaculture production [1]. However, like other agri-food sectors, it greatly impacts the environment. This is mainly due to the use of feeds for carnivorous fish commonly containing fishmeal and fish oil, raw materials no longer sustainable [2]. The colour and quality of fish livery and fillets can be influenced by nutrients such as carotenoids, omega-3, and ingredients derived from plants and algae present in their diet [3]. One of the objectives of the InsectFish project is to investigate the effects on fillet quality of partial substitution of fish meal with Black Soldier Fly larvae meal (BSFL meal, *Hermetia illucens*) in aquafeeds for Gilthead seabream (*Sparus aurata*). The project involves the collaboration of the University of Parma and the University of Pisa. A group of 131 gilthead seabream adults were experimentally farmed at the University of Pisa facilities for eight weeks until commercial size was achieved. A control diet (commercial) and an experimental diet containing a 10% BSFL meal as a partial substitution of the fish meal, was supplied to fish (2 treatments, 3 replicates). When slaughtered colour analysis on the livery and fillet of 33 fish per diet was recorded. A Minolta CR300 colourimeter (Illuminant D65) was used to measure lightness (L^*), redness (a^*) and yellowness (b^*) of the dorsal, caudal and ventral regions of the livery and fillets.

Repeated single ANOVA was performed to analyse each colour component (L^* , a^* and b^*) and their main effect between control and experimental, as well as for the different positions (D, C and V). The results showed no significant differences in the colour of fish's livery and fillets between the two diets. These findings suggest that including 10% BSFL meal does not influence the colour of Gilthead seabream, which is an important consideration for the consumer's perception of the product. The InsectFish project by the Next Generation EU call from the Italian Ministry of University and Research (MUR) PRIN 2022 PNRR (Progetti di Rilevante Interesse Nazionale 2022 Piano Nazionale di Ripresa e Resilienza).

[1] Food and Agriculture Organization (FAO), “The State of World Fisheries and Aquaculture 2024 - *Blue Transformation in Action*”, pp 266, 2024.

[2] Oliva-Teles, A., Enes, P., Couto, A., and Peres, H., “Replacing fish meal and fish oil in industrial fish feeds”. *Feed and Feeding Practices in Aquaculture*, pp 231-268, 2022.

[3] Pulcini, D., Capoccioni, F., Franceschini, S., Martinoli, M., and Tibaldi, E., “Skin pigmentation in gilthead seabream (*Sparus aurata* L.) fed conventional and novel protein sources in diets deprived of fish meal”. *Animals*, vol. 10(11), pp. 2138, 2020.

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Investigating the Microbiome of *Acheta domesticus* and *Locusta migratoria*-Based Food: A Preliminary Study

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The increasing global human population requires the introduction of sustainable alternative sources of nutrition. In Western countries, entomophagy is gaining interest, as edible insects are considered a sustainable source of dietary protein, with a lower ecological footprint than conventional protein sources. To date, the authorised production and marketing of Insect-Based Foods (IBFs) in Europe are limited to four species, that may be sold as whole insects, as powders or as ingredients in food products: *Tenebrio molitor* (yellow mealworm), *Locusta migratoria* (migratory locust), *Acheta domesticus* (house cricket), and *Alphitobius diaperinus* (lesser mealworm).

Despite their potential benefits, the introduction of IBFs into the European diet remains contentious, due to concerns that they may act as carriers of chemical hazards and opportunistic or pathogenic microorganisms, either originating from the insect gut or introduced via extrinsic sources such as farming conditions, handling, post-harvest starvation and rinsing, processing and preservation [1-4].

The aim of this preliminary study was to explore the microbial profiles of *Acheta domesticus* and *Locusta migratoria* IBFs available via European e-commerce platforms and to identify potential microbial risks, including spoilers and potential foodborne pathogens, using the 16S rRNA gene metabarcoding method.

Preliminary metabarcoding results revealed that, despite variations in food production and processing, IBFs showed a microbial signature characteristic of the insect species (*Acheta domesticus* vs *Locusta migratoria*). Furthermore, a diverse microbial community was identified, including both spoilage (*Lactococcus*, *Spiroplasma*, *Carnobacterium*, and *Streptococcus*) and potentially foodborne taxa (*Escherichia/Shigella*, *Staphylococcus*, *Bacillus* spp., *Salmonella* spp., and *Clostridia*).

In conclusion, the study highlights the potential of microbiome analysis as an emerging strategy for tracking insect species in IBFs and supporting the development of innovative food safety management plans in this novel and still largely unexplored insect supply chain.

[1] Frigerio, J., Agostinetto, G., Galimberti, A., De Mattia, F., Labra, M., & Bruno, A. (2020). Tasting the differences: Microbiota analysis of different insect-based novel food. *Food Research International*, 137, 109426

[2] Garofalo, C., Milanović, V., Cardinali, F., Aquilanti, L., Clementi, F., & Osimani, A. (2019). Current knowledge on the microbiota of edible insects intended for human consumption: A state-of-the-art review. *Food Research International*, 125, 108527

[3] Conway, A., Jaiswal, S., & Jaiswal, A. K. (2024). The potential of edible insects as a safe, palatable, and sustainable food source in the European Union. *Foods*, 13(3), 387

[4] EFSA, 2015. Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*, 2015;13(10):4257. <https://doi.org/10.2903/j.efsa.2015.4257>

Application of DNA Metabarcoding for the Botanical Traceability of Honey

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Honey is the third most adulterated food in the world after milk and olive oil, with mislabelling of geographical or botanical origin being a common occurrence. Consequently, the ability to verify the botanical traceability of honey is essential for food safety and fraud prevention. Adulteration and mislabelling not only mislead consumers but can also compromise the quality and authenticity of honey products. The official method for determining honey's botanical origin is based on the identification of pollen grains inside honey using light microscopy (melissopalynology), a time-consuming procedure that requires specialists in different plant groups. However, identification at the species or genus level frequently proves difficult, and melissopalynology has thus been coupled with DNA-based methods, particularly DNA metabarcoding [1]. The DNA metabarcoding approach relies on Next-Generation Sequencing (NGS) platforms, allowing for the simultaneous identification of all species present in complex matrices such as food products. This technique frequently achieves species-level identification and detects overlooked or unexpected plant taxa, thus improving the safety and reliability of honey authentication.

This pilot study applied DNA-metabarcoding of the ribulose-bisphosphate carboxylase (*rbcL*) gene to assess the floral composition of five honey samples from Apulia, Italy: one multifloral (Millefiori) and four monofloral (Chestnut, Cherry, Acacia, and Citrus).

Results confirmed the botanical species on labels while identifying additional taxa at lower abundances. These likely result from natural pollen transport by bees as co-flowering patterns suggesting environmental contamination rather than adulteration. The presence of a wide variety of pollens in the multifloral honey sample indicates that the bees foraged in an environment characterized by high floral biodiversity. Acacia honey has been found to contain predominantly *Quercus spp*, a result of the transportation process facilitated by honeybees [2]. No evidence of fraud was found in our dataset, which included only local honey products, confirming the high quality and safety of food products derived from a short supply chain.

These findings demonstrate the effectiveness of DNA-metabarcoding in accurately detecting the pollen composition of honey, enhancing product traceability and food safety [3]. The presence of trace plant species at low concentrations, which may result from cross-contamination during nectar collection, also suggests a potential use of botanical profiles to trace geographical origin, further strengthening the current food safety insurance and food fraud prevention strategies in the honey industry and facilitating accurate labelling for consumer protection.

- [1] A. Özkök *et al.*, “Comparing the melissopalynological and next generation sequencing (NGS) methods for the determining of botanical origin of honey,” *Food Control*, vol. 148, Jun. 2023, doi: 10.1016/j.food-cont.2023.109630.
- [2] S. Silici and M. Gökceoglu, “Pollen analysis of honeys from Mediterranean region of Anatolia,” *Grana*, vol. 46, no. 1, pp. 57–64, 2007, doi: 10.1080/00173130601138783.

- [3] C. Beltramo *et al.*, “Exploring the botanical composition of polyfloral and monofloral honeys through DNA metabarcoding,” *Food Control*, vol. 128, Oct. 2021, doi: 10.1016/j.foodcont.2021.108175.

Boosting Industry Transparency And Sustainability Through Portable Long-Read Sequencing Of Processed Seafood Products

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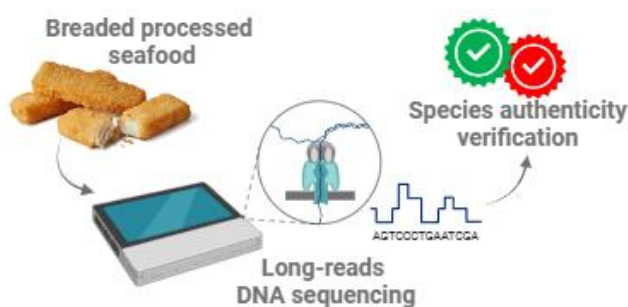


Figure 1 - Framework for species authenticity verification in processed seafood using a long-read DNA sequencing device

The processed seafood industry is a global network that involves various intermediaries, from fishermen and processors to distributors and retailers. This factor, along with the loss of diagnostic features along the production-supply chain, creates opportunities for unfair practices, such as the substitution or the addition of undeclared species, posing significant challenges in ensuring the authenticity and traceability of mixed seafood products throughout the supply chain [1]. Consequently, companies are called on to implement measures aimed at preventing food fraud and improving food safety. In recent years, the advent of high-throughput sequencing technologies, including second-

generation platforms (e.g., Illumina and Ion Torrent) and third-generation platforms (e.g., Pacific Biosciences and Oxford Nanopore Technology) has revolutionized the authentication of processed food matrices [2]. These advancements enable the simultaneous detection of multiple species within a single product, significantly enhancing analytical capabilities in food authenticity assessment. Among these, Oxford Nanopore Technology offers a cutting-edge approach to sequencing, characterized by its portability, real-time data generation, and ability to produce long reads [3]. However, to date, no study has utilized this sequencing technology as a tool for the authentication of commercial seafood products in Europe [4] [5]. Therefore, the aim of this study was to verify the species composition of 11 breaded seafood products, originating from a Dutch supplier that had declared single species composition for each product, ready to be packaged, labelled, and marketed in the Italian market by a regional distributor. DNA metabarcoding, which involved the amplification of long COI fragments (~650 bp) and the subsequent sequencing using the MinION device, revealed significant discrepancies in the species composition of these products (Figure 1). Specifically, in 7/11 products, undeclared species were detected, including flatfish, white fish and a freshwater species. In 3/11 products, the declared species was absent, while only 1/11 product exclusively contained the declared species. In conclusion, these findings underscore the mismanagement of raw material in the supplier processing plant, highlighting the need for robust authentication methods. The application of Oxford Nanopore Technology offers a viable solution for the seafood industry's authentication challenges, supporting its implementation as a promising tool for protecting consumers rights, ensuring species traceability and conservation efforts, and enhancing the integrity and safety of the seafood supply chain.

[1] Lorusso, L., Shum, P., Piredda, R., Mottola, A., Maiello, G., Cartledge, E. L., Neave, E. F., & Di Pinto, A., Mariani, S. (2024). Mismanagement and poor transparency in the European processed seafood supply revealed by DNA metabarcoding. *Food Research International*, 194, 114901.

[2] Haynes, E., Jimenez, E., Pardo, M. A., & Helyar, S. J. (2019). The future of NGS (Next Generation Sequencing) analysis in testing food authenticity. *Food control*, 101, 134-143.

[3] Rodríguez, M. D. S. T., Vanhollenbeke, J., & Derycke, S. (2023). Evaluation of DNA metabarcoding using Oxford Nanopore sequencing for authentication of mixed seafood products. *Food Control*, 145, 109388.

- [4] Ho, J. K., Puniemoorthy, J., Srivathsan, A., & Meier, R. (2020). MinION sequencing of seafood in Singapore reveals creatively labelled flatfishes, confused roe, pig DNA in squid balls, and phantom crustaceans. *Food Control*, 112, 107144.
- [5] Detcharoen, M., Khruakaew, P., Sukkapat, P., Benjakul, S., & Saetang, J. (2024). Metabarcoding for authentication of fish species in surimi-based products by Nanopore sequencing. *Food Bioscience*, 61, 104628.

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Bio-acidification for mitigating ammonia emissions in dairy barns: a preliminary laboratory study

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Ammonia (NH₃) emissions from livestock industry represent a significant risk for both human and animal health, and it has negative environmental impacts, such as contributing to particulate matter formation [1]. NH₃ emissions caused by manure management originate from nitrogen excreted by animals; it is released into the atmosphere when animal effluents are exposed to the external environment, mainly on farms, within manure storage areas. How reduction of greenhouse gases and air pollutants can be achieved is object of research; many scientific studies have focused on finding suitable mitigation strategies to limit the environmental impacts caused by the livestock sector [2-3].



Figure 1 - Manure samples collected in the barn

This study aims at investigating mitigation strategies to reduce NH₃ concentrations from the decomposition of animal manure in dairy cattle barns. In particular, the proposed bio-acidification strategy was based on the reduction of manure pH value in order to convert NH₃ into ammonium (NH₄), which, being less volatile, would reduce pollutant emissions into the atmosphere.

To this aim, the use of coffee silverskin, combined with acetic acid, has been tested for their bio-acidification effect in animal manure. Silverskin is a by-product of the coffee torrefaction process, very common in coffee consuming countries [4], yet not having any alternative uses in Sicily. Coffee silverskin and manure samples were collected both from the same area in Ragusa (Sicily, South Italy). Laboratory analyses were conducted by using various coffee silverskin and acetic acid treatments on manure, and subsequently, the pH changes in cattle manure samples were quantitatively assessed (see Figure 1). Low-cost instruments were utilised for pH measurement, and dedicated procedures were established for this purpose.

The sample with the best performance showed an average pH value reduction from 8 to 4.74. This bio-acidification approach, which utilises a by-product of the coffee industry, has the potential to offer an environmentally friendly, cost-effective and sustainable alternative to chemical acids. The findings of this research suggest that the utilisation of coffee silverskin as a bio-based manure management product could be a promising solution. In this direction, further investigation is required to assess this mitigation strategy in practical field conditions.

[1] Baldini, C.; Borgonovo, F.; Gardoni, D.; Guarino, M. Comparison among NH₃ and GHGs emissive patterns from different housing solutions of dairy farms. *Atmos. Environ.* 2016, 141, 60–66.

[2] Vitaliano S., D'Urso P. R., Arcidiacono C., Cascone G., 2024a, Ammonia Emissions and Building-Related Mitigation Strategies in Dairy Barns: A review, *Agriculture*, 14.

[3] Yan X, Ying Y, Li K, Zhang Q, Wang K (2024) A review of mitigation technologies and management strategies for greenhouse gas and air pollutant emissions in livestock production. *J Environ Manage* 352

[4] Narita Y, Inouye K (2014) Review on utilization and composition of coffee silverskin. *Food Research International* 61:16–22.

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Zebrafish from human to veterinary and animal sciences: a key model for measuring physiology, behaviour, and nutritional interventions

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Zebrafish (*Danio rerio*) has emerged as a prominent model in translational medicine, enabling the study of numerous human diseases. This species shares many genetic, physiological, and behavioural characteristics with other vertebrates [1,2]. The zebrafish model's unique features, including genetic similarity to humans, external fertilization, transparent embryos, and rapid development, make it an invaluable tool for understanding disease mechanisms, drug testing, and genetic research [3,4]. Recent developments have expanded its application into veterinary and aquaculture sciences, facilitating the study of fish health, welfare, and performance in laboratory conditions. This work aims to provide an overview of the current literature on the use of zebrafish in these research areas, focusing on two key aspects: (1) new methods for assessing fish physiology and welfare, and (2) the application of zebrafish in precision feeding for aquaculture.

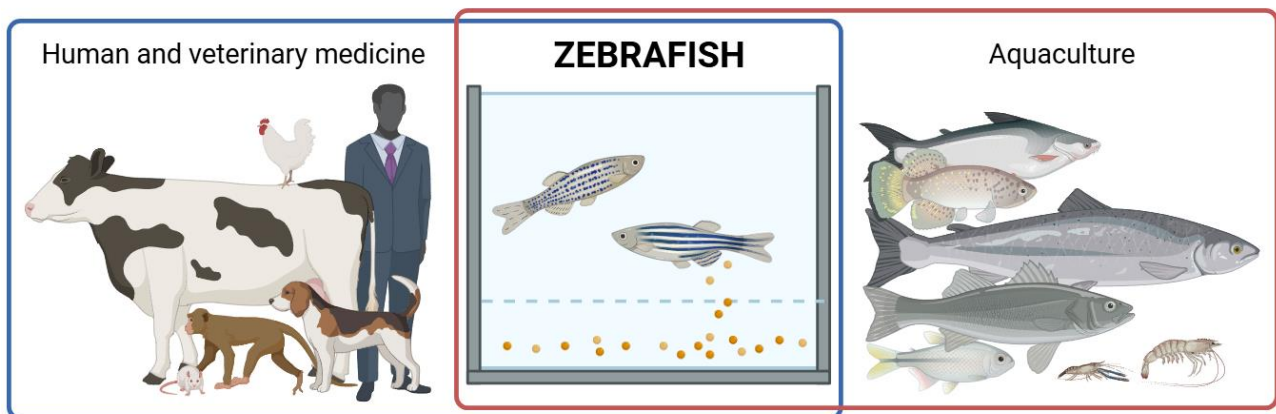


Figure 1 – The zebrafish model research areas

Recent advancements in non-invasive imaging and behavioural assays have enabled more accurate assessments of zebrafish welfare and health [5-8]. For example, fluorescence microscopy allows the visualisation of individual living cells and specific molecular markers, providing insights into organ function and physiological responses to environmental and nutritional variables. Behavioural assays, moreover, can detect changes in swimming patterns, stress responses and cognitive abilities. Precision feeding research has benefited from the use of zebrafish, which serve as a model for exploring nutritional interventions, analysing nutrient absorption, and optimising fish health and growth. These studies contribute to more sustainable and efficient fish farming practices [9,10].

Original data from the Department of Veterinary Sciences at the University of Pisa and other collaborating institutions are also presented, highlighting the physiological and behavioural responses of zebrafish to varying environmental and nutritional conditions. These findings reinforce the potential of zebrafish as a versatile model in both animal and human translational research, offering valuable insights for the study and management of other vertebrate species.

In conclusion, zebrafish represent a powerful tool in veterinary and animal sciences, particularly for assessing physiological and behavioural responses. Many of the technologies and insights developed through zebrafish research for human medicine are equally applicable to veterinary and animal science, further enhancing the potential of this model.

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- [1] Choi, T.Y., Choi, T.I., Lee, Y.R. *et al.* Zebrafish as an animal model for biomedical research. *Exp Mol Med*, 53, 310–317 (2021).
- [2] Rajanikant, G.K., Kalthur, G. Growing Importance of Zebrafish in Translational Neuroscience. In: Bhandari, P.R., Bharani, K.K., Khurana, A. (eds) *Zebrafish Model for Biomedical Research*. Springer, Singapore (2022).
- [3] Barros, T.P., Alderton, W.K., Reynolds, H.M., Roach, A.G. Berghmans, S. Zebrafish: an emerging technology for *in vivo* pharmacological assessment to identify potential safety liabilities in early drug discovery. *British Journal of Pharmacology*, 154: 1400-1413 (2008).
- [4] Norton, W., Bally-Cuif, L. Adult zebrafish as a model organism for behavioural genetics. *BMC Neurosci*, 11, 90 (2010).
- [5] Chan, P.K., Lin, C.C. Cheng, S.H. Noninvasive technique for measurement of heartbeat regularity in zebrafish (*Danio rerio*) embryos. *BMC Biotechnol*, 9, 11 (2009).
- [6] Zhan, T., Song, W., Jing, G. *et al.* Zebrafish live imaging: a strong weapon in anticancer drug discovery and development. *Clin Transl Oncol*, 26, 1807–1835 (2024).
- [7] Egan, R.J., Bergner, C.L., Hart, P.C., *et al.* Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behav. Brain Res*, 205(1), 38–44 (2009).
- [8] Miklósi, A., Andrew, R.J. The zebrafish as a model for behavioral studies. *Zebrafish*, 3(2), 227–234 (2006).
- [9] Williams, M.B., Watts, S.A. Current basis and future directions of zebrafish nutrigenomics. *Genes Nutr*, 14, 34 (2019).
- [10] Watts, S. A., Lawrence, C., Powell, M., D'Abramo, L. R. The Vital Relationship Between Nutrition and Health in Zebrafish. *Zebrafish*, 13(1), S72–S76 (2016).

APPàre: The Digital Platform for Innovation in the Sardinian Livestock Sector

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Figure1. APPàre platform ecosystem

Introduction:

Data is the currency to boost data-driven innovation in EU food systems. However, in extensive livestock system digital innovation and data collection, sharing and analysis are still lacking. The main challenge in this sector is not the lack of data itself but rather that data are often not systematically analysed, nor integrated with other relevant information, thus hampering their potential to generate meaningful insights. Arising from this need, the APPàre platform was developed, aiming to create a platform for data collection, management, and analysis. This platform is designed to provide valuable insights and decision-support tools for the dairy sheep farming sector, enhancing efficiency and sustainability across the supply chain.

Materials and methods:

The APPàre platform, developed involving the end users in the creation process, provides herders and farm's practitioners with a powerful tool that allows to record, store, and analyse all farm data and flags any risk/weakness found in the productive chain. It accepts data from different sources, such as: external databases, data from sensors and devices, user inserted data. The collected data are aggregated and processed, to visualise on the dashboard relevant indexes and suggestions. As for example, a mobile application enables farm veterinarians to record reproductive data directly via their smartphone. At the end of the veterinary check, the application immediately generates a summary report with a descriptive statistic of the data from the flock and integrates their analyses with other relevant information (i.e., health and physiological status, environmental variables, feeding, etc...).

Preliminary Results:

The preliminary results are those related to promote processes of integrated innovation and technology transfer, enabling herders to manage their businesses with the developed platform, and at the same time providing useful feedback for its improvement. This would provide a clear and advanced overview of what will happen in the near future, enabling herders to strategically position themselves in the market and plan their production processes accordingly. Meanwhile veterinarians would have the benefit of a comprehensive and continuously updated health record for each farm, facilitating more precise monitoring of animal welfare, productions and health status. This systematic approach would enhance herd health management, enabling veterinarians to implement evidence-based interventions and collaborate with farmers in optimizing husbandry practices and decision-making processes to improve overall farm sustainability and productivity. Having a broader perspective, this platform could also be utilized by policymakers to shape health and animal welfare policies, as well as regional agricultural policies.

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Impact of cold stress on daily milk production in Italian Mediterranean Buffaloes

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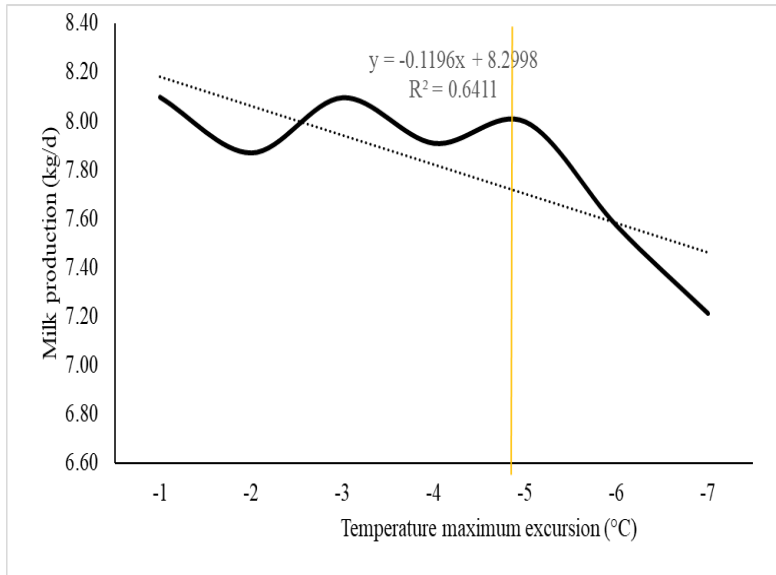


Figure 1 – Effect of temperature maximum excursion on daily milk production.

The increasing frequency of extreme weather events due to climate change poses challenges to dairy buffalo farming, potentially reducing milk production. While extensive research has examined the effects of heat stress on dairy cows, limited studies have addressed the impact of cold stress on lactating buffaloes. Indeed, buffaloes may experience physiological stress under cold conditions. This study aims to assess the influence of cold stress on daily milk production in Mediterranean Italian buffaloes. A total of 60 lactating buffaloes, comprising 40% primiparous and 60% second-parity animals, were monitored within a single herd from September 2023 to May 2024. Daily milk production was recorded using a DeLaval milking robot (VMS 300), while temperature, humidity, and wind speed were measured every 15 minutes by the Davis Pro 2 weather

station. All data were transmitted, processed, and stored on a centralised server for analysis using a Python pipeline developed by the authors. To quantify environmental stressors, the Temperature-Humidity Index (THI) was calculated modifying the formula: $THI = (1.8T + 32) - [(0.55 - 0.0055H)(1.8T - 26)]$. To better represent cold stressor events, THI was calculated using daily minimum temperature and maximum relative humidity. Additionally, the Windchill Index (WHI) was computed to account for the combined effects of wind speed and temperature: $WHI = 33 - (33 - T)(0.47 + 0.45\sqrt{Wind\ speed} - 0.05\ Wind\ speed)$. The relationship between these indices and daily milk production was analysed using a repeated mixed model: $y_{ijklm} = Parity\ order_i + DIM_j + Month_k + Index_l + Animal_m + Error_{ijklm}$, where $Index_l$ represents either THI or WHI. Furthermore, a separate model was developed to evaluate the effect of negative thermal excursions, on milk yield, incorporating the decrease in maximum daily temperature between consecutive days as a fixed effect. The results indicated a trend toward reduced milk yield when THI dropped below 58 ($P = 0.08$) and a significant decline when WHI exceeded 14 m/s ($P = 0.04$), highlighting the detrimental impact of cold stress and wind exposure. Additionally, thermal excursion exceeding 5°C between consecutive days exacerbated milk yield reduction, with a progressive decline observed as temperature fluctuation increased. These findings underscore the necessity of integrating environmental indices into farm management strategies to enhance buffalo welfare and productivity, particularly in temperate climates where cold stress is often underestimated. Further research incorporating multi-year datasets and larger sample sizes is warranted to refine predictive models and improve resilience in dairy buffalo farming under increasingly variable climatic conditions.

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Evaluation of Coccimorph image-based software as a complementary tool for the identification of chicken *Eimeria* species in field samples

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Eimeria spp. are common parasites of chickens, causing important health problems and production losses [1]. The ten *Eimeria* species infecting chickens are characterized by different pathogenicity [2, 3].

Microscopic identification of chicken *Eimeria* spp. is unreliable due to overlapping morphological features [4]. Polymerase chain reaction (PCR)-based assays also face challenges and the routine application of PCR in field samples remains limited [5]. Coccimorph is an on-line image-based software which allows the identification of *Eimeria* species based on oocyst curvature, size, symmetry and internal structure quantification [6].

This study aimed to evaluate Coccimorph as a complementary tool for *Eimeria* species identification in field samples positive for mixed species.

Images (40x and 100x magnification) of purified sporulated oocysts of *Eimeria tenella* (n. 25), *Eimeria acervulina* (n. 25), and *Eimeria maxima* (n. 25) and 100 images of individual sporulated oocysts randomly taken from 13 field faecal samples, were uploaded to the software and the identified *Eimeria* species were recorded. All oocysts were also identified using a PCR assay [5]. Data were statistically analysed using the χ^2 test (GraphPad Prism Ver. 8.4.2).

Eimeria maxima, *E. acervulina* and *E. tenella* purified oocysts were identified by Coccimorph in 88.0%, 80.0% and 72.0% of the cases, respectively. In field samples, the agreement of *Eimeria praecox*, *Eimeria necatrix*, *E. tenella*, *E. acervulina* and *Eimeria brunetti* identification by the software and PCR was 84.2%, 71.4%, 64.3%, 63.6%, and 50.0%, respectively. No statistically significant differences were found between Coccimorph and PCR results. However, *Eimeria mitis* was detected by Coccimorph but not by PCR-assay. Lower magnification and resolution images were more efficiently processed by the software.

Good agreement between PCR and Coccimorph identification has been reported previously for *E. acervulina* and *E. mitis* [5]. In this study, a good agreement was found for *E. praecox* and *E. necatrix*. Agreement fared less well with *E. acervulina*, *E. tenella* and *E. brunetti* and no agreement was observed for *E. mitis*, probably due to the low mean number of *E. mitis* oocysts per gram of faeces (324 OPG) in the analysed samples, as PCR sensitivity decreases significantly for samples with ≤ 500 OPG [5].

Overall, Coccimorph proved a useful complementary tool in field mixed-species infections. However, it does not include three newly described chicken *Eimeria* species [3] and results may depend on image quality.

[1] Blake et al. Vet. Res. vol 51, pp. 1-14, 2020.

[2] López-Osorio et al. Front. Vet. Sci vol 7, pp. 384, 2020.

[3] Jaramillo-Ortiz et al., Vet Parasitol. vol 324, 110068, 2023.

[4] Haug et al. Avian Pathol. vol 37, pp. 161-70, 2008.

[5] Kumar et al. Vet Parasitol. Vol 199, 24-31, 2014.

[6] Castañón, et al. Pattern Recognition vol. 40, pp. 1899-1910, 2007.

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Optimizing Current Approach to Control Cystic Echinococcosis using Drone Technology and geospatial tools

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Figure 1. Flying over the flock: aerial images for research on Cystic Echinococcosis in Southern Italy.

Cystic Echinococcosis (CE) is a zoonotic parasitic disease posing significant threats to global human and animal health. CE is recognized by the World Health Organization (WHO) as one of the 21 neglected tropical diseases (NTDs) and is prioritized in the WHO's Roadmap for 2030. Therefore, there is an urgent need for effective strategies and research into new technologies and tools to control this infection in areas endemic for CE such as southern Italy [1]. This study aims to optimize control strategies against CE through the use of advanced technologies, with a particular focus on the use of GPS dataloggers and drones [2,3]. The collection of detailed data on the movements and spatial behavior of definitive (e.g. dogs) and intermediate hosts (e.g. sheep) is crucial for planning effective control strategies against CE in endemic areas. Geospatial technologies and GPS dataloggers

offer an ideal solution for tracking animal movements with high spatial and temporal accuracy to identify the most frequented grazing areas (Figure 1). This approach is useful to estimate high-risk areas for canid access around positive farms to propose a scheme for delivering praziquantel-laced baits to treat unowned dogs freely roaming near grazing areas. In addition, integrated drone-based systems were recently implemented for the delivery PZQ-laced baits to deworm stray canids in the vicinity of small ruminant grazing area. The system consisted of a DJI Flame wheel F550 drone equipped with a 3D-printed mechanical dispenser [4]. The dispenser was connected to a servo motor that enabled its rotation and the placement of baits specifically prepared by the veterinarians for the treatment. The onboard camera enabled a detailed visualization and identification of the precise location for bait release in the field. The dispenser was connected to a servo motor that enabled its rotation. The motor's movements were controlled by a STM32F401 Nucleo-64 microcontroller, specifically programmed to carry out the required operations. This system was designed to minimize the waste of resources and quickly deliver the antiparasitic treatment to hard-to-reach areas. It is emphasised that a multi-disciplinary and multi-institutional One Health effort is fundamental for the development of CE control programmes at different geographic levels.

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- [1] G. Cringoli, P. Pepe, A. Bosco, M.P. Maurelli, L. Baldi, P. Ciaramella, V. Musella, M.L. Buonanno, F. Capuano, F. Corrado, D. Ianniello, L.C. Alves, P. Sarnelli, and L. Rinaldi, "An integrated approach to control Cystic Echinococcosis in southern Italy," *Vet. Parasitol.*, vol. 290, pp. 109347, 2021.
- [2] E. Ciccone, A. Bosco, P. Pepe, M. Nocerino, N. Lattero, G. Umhang, L. AbdElkarim, S. Lahmar, Y. Said, G. Saralli, G. Piegari, M.C. Alterisio, R. Baka, S. Sotiraki, F. Boué, and L. Rinaldi, "Baiting not-owned dogs against *Echinococcus granulosus*: innovative tools for integrated control," *Parasitology*, vol. 151, pp. 421-428, 2024.
- [3] M. Nocerino, P. Pepe, A. Bosco, E. Ciccone, M.P. Maurelli, F. Boué, G. Umhang, J. Pellegrini, S. Lahmar, Y. Said, S. Sotiraki, P. Ligda, A. Laatamna, G. Saralli, O. Paciello, M.C. Alterisio, and L. Rinaldi, "An innovative strategy for deworming dogs in Mediterranean areas highly endemic for cystic echinococcosis," *Parasit. Vectors*, vol. 17, pp. 86, 2024.
- [4] E. Caputo, G. de Alteriis, C. Conte, M. Nocerino, P. Pepe, S. Elia, A. Bosco, G. Cringoli, L. Rinaldi, G. Rufino, and D. Accardo, "Development of an Embedded System-Based Dropper Payload for Drones," *IEEE 9th International Workshop on Metrology for AeroSpace, MetroAeroSpace 2022 – Proceedings*, vol. 2022, pp. 639–643, 2022.

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Open-source toolkit for in vitro diagnosis of malaria tailored for low-resource settings

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Malaria, a life-threatening disease caused by *Plasmodium* protozoans and transmitted by female *Anopheles* mosquitoes, accounted for 263 million cases and 597,000 deaths in 2023 [1]. Microscopy remains the gold standard for diagnosis for its ability to identify the species and quantify parasitaemia. However, its reliance on laboratory equipment and skilled personnel limits implementation in low-resource settings (LRS) [2]. To overcome these challenges, a diagnostic toolkit (a smearer, a staining jar with a filter, an image system, and a quality assessment pipeline, figure 1) was developed using UBORA platform and employing 3D printing technique to enable for local manufacturing and improve accessibility. The devices were designed to comply with context-sensitive requirements, extracted from an on-field study conducted in Kenya. A blood thin smearer for producing high-quality thin blood smears was developed and validated at Pisa University Hospital’s Haematology Unit, where device performances were assessed comparing a minimally trained non-specialized operator, the prototype device, and the commercial Sysmex SP 10 smearer. The evaluation involved ten thin smears from a healthy blood donor, stained with Giemsa for clear visualization of red blood cells under an optical microscope. Both macroscopic and microscopic analyses were performed, alongside an assessment of the time required for each method. The quality was assessed with a pipeline developed in Python [3]. The prototype demonstrated compliance with high-quality smear criteria in macroscopic evaluation, and its microscopic performance was found to be comparable to the commercial device while better adapting to the constraints of LRS. A staining jar was designed to regulate the volume of staining solution used. Three membrane-less filtration system (straight channel, snake channel, and manta ray channel) were simulated using finite element modelling in COMSOL Multiphysics. Among these, the snake channel filter exhibited the highest filtration efficiency. The filter was then manufactured using a stereolithography printer and underwent preliminary testing to evaluate its performance. To complete the malaria diagnostic process, a prototype of an open-source movement stage equipped with a DIPLE lens (Smart MicroOptics, Italy) was designed and manufactured. This system allows image acquisition using a mobile phone camera, with a design optimized for 3D printing to simplify maintenance and reduce reliance on specialized commercial components [3].

Overall, the toolkit represents significant progress in malaria diagnostics, particularly in LRS, where accessibility, cost-effectiveness, and ease of use are critical factors. It has the potential to enhance diagnostic accuracy and efficiency, contributing to better disease management and reducing malaria-related mortality.

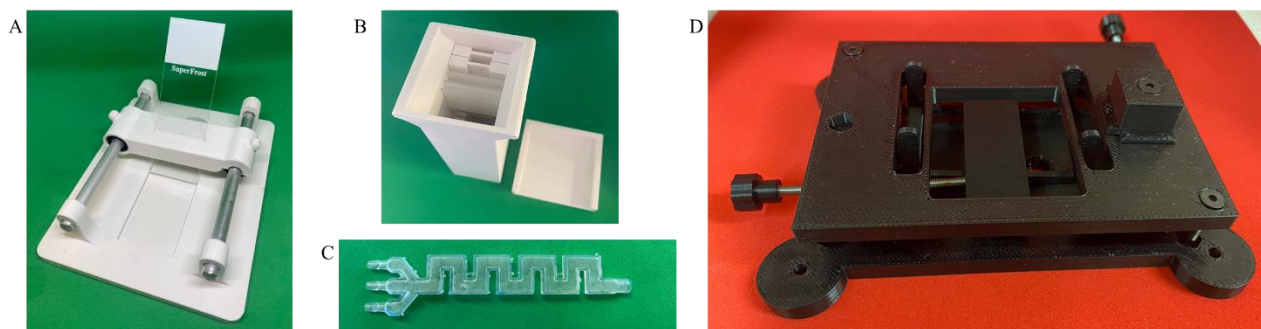


Figure 1 – A: Blood thin smearer; B: Staining Jar; C: Snake-like filter; D: Stage for image acquisition.

[1] World Health Organization. (2024). World malaria report 2024.

[2] Coro, F., Arcangeli, A., De Maria, C., Mangano, V., & Ahluwalia, A. (2023, September). Open-Source Medical Device for in Vitro Diagnosis of Malaria. In Mediterranean Conference on Medical and Biological Engineering and Computing (pp. 208-214). Cham: Springer Nature Switzerland.

[3] Coro, F., Mangano, V., Ahluwalia, A., & De Maria, C. (2025). Open-source toolkit for image acquisition and quality assessment of thin blood smears for malaria diagnosis. Biomedical Signal Processing and Control, 103, 107470.

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Integrating engineering and public health to monitor waterborne intestinal pathogens: a one health approach

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Figure 1: Aerial photo of a flood-affected town center in a developed country [1].

Intestinal pathogens (e.g. *Campylobacter*, *Cryptosporidium* and *Giardia*) are responsible for waterborne infections causing diarrheal diseases in animals and humans even in high-income countries [2]. Water plays a significant role in their transmission, mainly as consequence of the runoff from agricultural fields and livestock farms, due to the discharge of wastewater treatment plants, which are not regulated by the current legislation [3]. International mobility, urban expansion, proximity to livestock farming, and poor hygiene practices increase the risk of human exposure to these pathogens [4]. Additionally, extreme climatic events can exacerbate water contamination and overload sanitation systems, further amplifying the incidence of bacterial and parasitic infections [5]. Within this context, a multidisciplinary project has been funded by the EU (Horizon Europe) to understand and mitigate the risks that climate change

poses to microbial water quality and to the prevalence of waterborne diarrheal infections. The study is centered on four case studies referred to four research areas across Africa and Europe: Accra (Ghana), Haydom (Tanzania), Timisoara (Romania), and Naples (Italy). Each case study presents distinct characteristics in terms of socioeconomic factors, urbanization rate, climate risk, and healthcare system vulnerability.

An integrated approach will allow to achieve several key objectives: managing potential biological risks associated with potable water supply and wastewater disposal; conducting geospatial contamination surveys using Geographic Information Systems; modeling the impact of global climate change on local hydro-climatic variables and human health; predicting the disease spread using models validated by the surveillance data collected; testing mitigation strategies under various climatic, health, and developmental scenarios.

The synergy between engineering sciences and public health will allow the monitoring of zoonotic infections of human and veterinary interest, through digital image processing models and pattern recognition techniques within a One Health perspective. This project aims to develop water safety plans in which synergistic interventions for adaptation to climate shocks and the limitation of diarrheal diseases will be contained. Finally, the collaboration between governmental authorities, technical infrastructure operators, public health professionals, and local communities will align with global sustainable development goals focused on water, sanitation, and health. This will ensure that drinking water remains safe from source to consumption, guaranteeing supply even under risk conditions and preventing contamination.

[1] <https://nypost.com/wp-content/uploads/sites/2/2022/07/kentucky-river-flooding-3.jpg>

[2] L. Andrade, J. O'Dwyer, E. O'Neill, and P. Hynds, "Surface water flooding, groundwater contamination, and enteric disease in developed countries: A scoping review of connections and consequences", *Environ. Pollut.*, vol. 236, pp. 540-549, 2018.

[3] N.A. Moreira and M. Bondelind, "Safe drinking water and waterborne outbreaks", *J. Water Health*, vol. 15(1), pp. 83-96, 2017.

[4] J. Wolf, R.B. Johnston, A. Ambelu, B.F. Arnold, R. Bain, M. Brauer, J. Brown, B.A. Caruso, T. Clasen, J.M. Colford Jr, J.E. Mills, B. Evans, M.C. Freeman, B. Gordon, G. Kang, C.F. Lanata, K.O. Medlicott, A. Prüss-Ustün, C. Troeger, S. Boisson, and O. Cumming, "Burden of disease attributable to unsafe drinking water, sanitation, and hygiene in domestic settings: a global analysis for selected adverse health outcomes", *Lancet*, vol. 401(10393), pp. 2060-2071, 2023.

[5] J.C. Semenza and A.I. Ko. "Waterborne Diseases That Are Sensitive to Climate Variability and Climate Change", *N. Engl. J. Med.*, vol. 389(23), pp. 2175-2187, 2023.

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Innovative technologies for diagnosis of intestinal parasites in animals and humans

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Figure 1. Kubic FLOTAC Microscope

The intestinal parasites remain a significant global veterinary and public health concern, with substantial socio-economic impacts [1,2]. In recent years, remarkable progress has been made in copromicroscopic diagnostic field thanks to the development of new digitized (semi-) automated tools that enable the specific, sensitive, accurate, precise, rapid, user-friendly, standardized and reliable recognition of parasitic elements, as well as the assessment of Faecal Egg Count/Faecal Egg Count Reduction (FEC/FECR) for both pen-side and laboratory use. This new generation of diagnostic systems equipped with Artificial Intelligence (AI) algorithms, such as machine learning and deep learning, has been developed to overcome gaps and limitations of traditional microscopy (i.e., human errors and time for analysis) in veterinary and human field. For this reason, through collaboration among Dept. of Veterinary Medicine and Animal Production, Dept. of Electrical Engineering and Information Technology and a company of Mechanical Engineering, a new automated system for diagnosis of helminth eggs in animals and humans was developed, the Kubic FLOTAC Microscope (KFM). The KFM

is a compact, low-cost digital microscope that combines the high sensitivity, accuracy and precision of the Mini-FLOTAC/FLOTAC techniques with a reliable system based on AI predictive model. After capturing high resolution pictures, the system is able to recognize and count the parasitic elements in the analysed samples. This system can be remotely controlled via software by smartphone, tablet or PC, and via internet it is possible to transfer the captured pictures to other laboratories, that could be very useful to create a network or to support operators directly in the field [3]. A dataset with more than 5,000 objects for each analysed parasite was used. The acquired images are first segmented into background and objects, which can be either impurities or pseudo-parasites with the main goal of being able to characterize the parasite species and to automatically count eggs. The AI system is able to recognize the 90% of the parasitic elements analysed [4]. Therefore, the KFM is a promising automated system for a rapid and accurate assessment of FEC to improve the diagnosis of parasitic performing an effective and efficient treatment in a short time and assisting a new generation of operators in veterinary and human parasitology.

[1] J. Charlier, D.J. Bartley, S. Sotiraki, M. Martinez-Valladares, E. Claerebout, G. von Samson-Himmelstjerna, S.M. Thamsborg, H. Hoste, E.R. Morgan, and L. Rinaldi, "Anthelmintic resistance in ruminants: challenges and solutions". *Adv. Parasitol.*, vol. 115, pp. 171–227. 2022.

[2] World Health Organization, "Soil-transmitted helminth infections. World Health Organization, Geneva, Switzerland. Available from: <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>

[3] G. Cringoli, A. Amadesi, M. P. Maurelli, B. Celano, G. Piantadosi, A. Bosco, L. Ciuca, M. Cesarelli, P. Bifulco, A. Montresor, L. Rinaldi, "The Kubic FLOTAC Microscope (KFM): a new compact digital microscope for helminth egg counts", *Parasitology*, vol. 148 (4), pp. 427–434, 2021.

[4] S. Capuozzo, S. Marrone, M. Gravina, G. Cringoli, L. Rinaldi, M. P. Maurelli, A. Bosco, G. Orrù, G. L. Marcialis, L. Ghiani, S. Bini, A. Saggese, M. Vento, C. Sansone, "Automating parasite egg detection: insights from the first AI-KFM challenge", *Front. Artif. Intell.*, vol. 7, pp. 1-17. 2024.

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Risk assessment of (re)-emerging Vector-Borne Diseases in Southern Europe based on Machine Learning processing of poly-observational data

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Mosquito-borne diseases are a major global public health concern, with arboviruses and filarial parasites posing substantial risks to humans and animals [1]. In Mediterranean regions, including Italy and Greece, mosquito distribution and population dynamics are influenced by ecological, environmental, and anthropogenic factors [2]. Among the most prominent vectors are *Culex pipiens* and *Aedes albopictus*, which transmit diseases like West Nile virus (WNV), Usutu virus (USUV), and filarial nematodes [3]. Understanding the spatiotemporal distribution and ecological drivers shaping these mosquito populations is critical for effective vector control and disease prevention strategies.

This study integrates anthropogenic, environmental, and genetic factors to investigate mosquito ecology and disease transmission at regional and microclimate scales. Focusing on two Mediterranean regions in Central Italy (Marche and Umbria) and central Crete (Heraklion and Rethymnon), it provides a comparative framework to assess vector ecology across diverse socio-environmental contexts.

A comprehensive analysis of mosquito population dynamics and pathogen circulation was conducted across the biennium 2022-2023, emphasizing the influence of ecological and anthropogenic factors on vector distribution and transmission risk. To identify key drivers shaping mosquito populations, machine learning techniques—specifically the Self-Organizing Map (SOM), a type of artificial neural network—were applied.

The SOM was trained on ecological data from Marche and Umbria collected in 2022, analysing the distribution of *Aedes albopictus* and *Culex pipiens* based on 42 environmental variables, including climate and land cover types. Using a 9×9 hexagonal grid, SOM effectively clustered the two species, revealing distinct ecological associations: *Ae. albopictus* was strongly linked to urbanized and human-modified environments, while *Cx. pipiens* exhibited broader adaptability to agricultural and semi-natural habitats. These findings underscore the role of ecological factors in shaping mosquito distributions. SOM model provides valuable insights, but variations in predictive performance highlight the need for further refinement to improve accuracy and address dataset limitations.

Nevertheless, our study lays the foundation for developing predictive models tailored to regional environmental conditions, enhancing vector surveillance and control strategies. Integrating multiple surveillance parameters into a unified analytical framework, it contributes to a more precise, data-driven approach to mosquito management, optimizing control measures for specific regional contexts. Future advancements in dataset integration and SOM refinement will improve region-specific vector control strategies, enabling more targeted interventions. The incorporation of anthropogenic, environmental, and genetic variables into a comprehensive surveillance system represents a significant step forward in mitigating the risk of mosquito-borne diseases, ultimately strengthening public health preparedness and response in the Mediterranean region.

- [1] Valenzuela JG, Aksoy S. Impact of vector biology research on old and emerging neglected tropical diseases. PLoS Negl Trop Dis. 2018 May 31;12(5):e0006365.
- [2] Brugueras S, et al. Environmental drivers, climate change and emergent diseases transmitted by mosquitoes and their vectors in southern Europe: A systematic review. Environ Res. 2020 Dec;191:110038.
- [3] Logiudice J, et al. Introduction of Vector-Borne Infections in Europe: Emerging and Re-Emerging Viral Pathogens with Potential Impact on One Health. Pathogens. 2025 Jan 12;14(1):63.

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The use of ruminal boluses to detect bouts in beef cattle

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Monitoring drinking behaviours and water consumption in cattle is crucial for ensuring health and well-being. Farming technologies such as reticular boluses manufactured by smaXtec animal care GmbH (<https://smaxtec.com>) not only allow

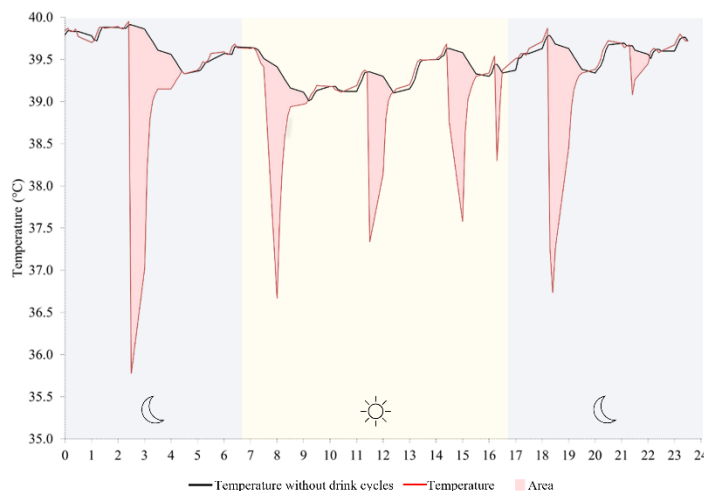


Figure 1. Example of longitudinal measurement of the reticular temperature to detect drinking episodes (red peaks) and their magnitude (area under the curve). The blue portions represent the nocturnal phases, while the yellow portion represents the diurnal phase.

for monitoring the overall health status of the herd thanks to specific alerts, but they can also be used to develop customized monitoring tools, further enhancing both animal welfare and farm efficiency [1].

This study aims to develop an algorithm to detect number and magnitude of drinking episodes in beef cattle by processing data recorded by individual boluses of smaXtec animal care GmbH (<https://smaxtec.com>). Boluses are orally administered using a specialized applicator and they pass through the oesophagus and settle in the reticulum, where they remain to monitor various parameters at 10-minute intervals, including temperature (°C) and temperature without drinking cycles (°C). The bolus wirelessly transmits data to a base station that stores data in the manufacturer's cloud for a preliminary correction before alerts production.

A total of 181,323 longitudinal temperature observations coming from 60 animals were collected

over a period of 21 days, from October 22, 2021, to November 12, 2021. The data were processed, edited and analysed using the R software, version 4.3.2 [2]. The algorithm developed works by comparing the temperature differences between non-drinking cycles and bolus transmissions. In particular, each drinking episode is recorded when the difference fell below a set threshold which can be each time modified on a voluntary basis (Figure 1). The algorithm also calculates the area under the curve to assess the magnitude of each episode on a daily basis (daytime and night; Figure 1) and it can potentially indirectly provide the amount of water ingested but reference water intake data should be used to develop prediction model (Figure 1). Results demonstrated that bouts can be detected via longitudinal data analysis. In addition, in the study trial the animals tended to drink more frequently during the night, but they drunk more during the day (5.18 ± 2.08 L) than night (2.52 ± 1.51 L).

In conclusion, although the algorithm has a certain potential to predict number of bouts and water intake at each bout, at the moment it does not allow for a direct measurement of water intake because reference data are still not available at individual level. Nevertheless, it still serves as an indicator of the number and magnitude of each episode since a larger area suggests a greater temperature drop, which correlates with higher water consumption. Further studies may improve the algorithm and assess the boluses' ability to predict water-related features in both beef and dairy cattle.

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[1] Vázquez-Diosdado, J. A., et al. "Developing and evaluating threshold-based algorithms to detect drinking behaviour in dairy cows using reticulo-rumen temperature." *Journal of Dairy Science* 102.11 (2019): 10471-10482.

[2] R version 4.3.2 (2023-10-31)

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Heart Girth as a Predictor of Body Weight in Lactating Cows

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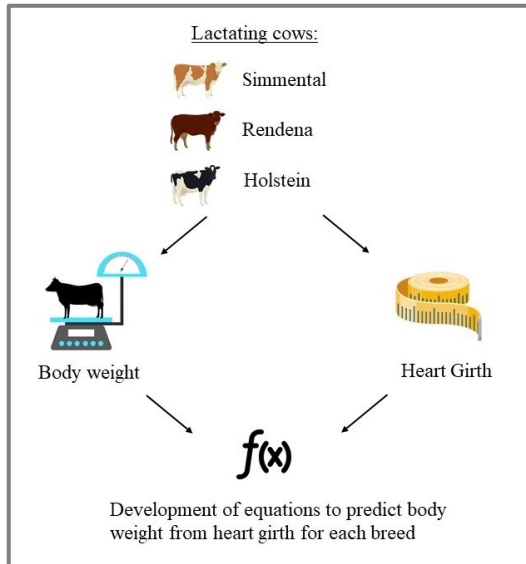


Figure 1 - Experimental design and rationale of the study.

Body weight of cows is a relevant morphological trait as it is commonly used in the literature to predict other variables such as dry matter and water intake, urine excretion and methane emissions [1, 2]. Measuring body weight on a large scale in dairy cows is not easily feasible and the literature reports equations to predict body weight based on body measurements (i.e., heart girth, wither height, hip width and body length) in Holstein heifers [3, 4]. In particular, prediction of body weight from heart girth has been shown to be more accurate than other morphometric measurements [3]. To our knowledge, no studies have attempted to develop equations to predict body weight of dairy and dual-purpose lactating cows. Therefore, the aim of this study is to develop equations to predict body weight from heart girth in lactating Holstein, Simmental and Rendena cows.

Between February and July 2024, 293 lactating cows (94 Holstein, 52 Simmental and 147 Rendena) were selected in 6 farms located in the northern Italy. On the same day, heart girth (the circumference around the thorax at the level of the heart) was measured for each cow using a weight tape and the average of three body weight measurements from the scale of automatic milking system was recorded (Figure 1).

For each breed, linear, quadratic and cubic regressions of body weight on heart girth were performed and coefficients of determina-

tion (R^2) and standard error reported.

The heart girth averaged 212.40 ± 8.80 cm, 210.90 ± 10.31 cm, and 199.50 ± 8.47 cm for Holstein, Simmental and Rendena cows, respectively. Meanwhile, the average body weight was 678.80 ± 75.94 kg for Holstein, 712.20 ± 78.05 kg for Simmental, and 642.10 ± 63.67 kg for Rendena. The R^2 values from the regressions showed a strong correlation between body weight and heart girth in all the breeds studied, with the R^2 being greater in cubic regression than in the other types of equations. This study demonstrates that heart girth is a reliable predictor of body weight in lactating Holstein, Simmental and Rendena cows.

- [1] X.B. Chen. "Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives-An overview of the technical details", *International Feed Resources Unit, Rowett Research Institute*, 21. 1992.
- [2] A. Bougouin, J.R.N Appuhamy, A. Ferlay, E. Kebreab, C. Martin, P.J Moate, C. Benchaar, P. Lund and M. Eugène, "Individual milk fatty acids are potential predictors of enteric methane emissions from dairy cows fed a wide range of diets: Approach by meta-analysis", *Jour. Dairy Sci*, vol 102, pp.10616-10631, 2019.
- [3] A.J. Heinrichs, G.W. Rogers, and J.B. Cooper, "Predicting body weight and wither height in Holstein heifers using body measurements", *Jour. Dairy Sci*, vol. 75, pp. 3576-3581, 1992.
- [4] A.J. Heinrichs, B.S. Heinrichs, C.M. Jones, P.S. Erickson, K.F. Kalscheur, T.D. Nennich, B.J. Heins, and F.C. Cardoso, "Verifying Holstein heifer heart girth to body weight prediction equations", *Jour. Dairy Sci*, vol. 100, pp. 8451-8454, 2017.

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Using Solomon Coder for the Ethological and Feeding Behaviour Analysis of Dairy Cows in Tie-Stall Housing: a Practical Guide

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Evaluating the behaviour of dairy cows is a key element in ensuring animal welfare and optimizing farm management strategies [1]. Ethological analysis provides valuable insights into the daily activities of animals, helping to identify potential welfare issues and develop evidence-based solutions. Reliable and user-friendly tools are therefore essential for collecting and analysing behavioural data [2]. This study outlines the use of Solomon Coder (<https://solomoncoder.com>)

as a support tool for ethological analysis, with a particular focus on feeding behaviour. It offers a practical guide for managing and processing behavioural observations gathered through video recordings (Fig.1).



Figure 1 - Screenshot of the setup used for behavioural observation, showing the fixed camera recording a dairy cow and the Solomon Coder interface. The system allows for precise categorization and analysis of various activities

The data were collected using a fixed camera that recorded a single cow over six consecutive days, resulting in 144 one-hour videos. This continuous monitoring provided a detailed picture of the cow's behaviour throughout the day, enabling a precise time-budget analysis. The observed behaviours included: eating TMR, eating pelleted concentrate, ruminating while standing, ruminating while lying down, standing idle, resting, drinking, social interactions, self-grooming, and milking (not visible in the video as it occurred at the milking robot).

Solomon Coder was then used for the creation of behavioural categories and for recording and processing the data. The results showed that this tool provides clear and detailed outputs, enabling quick and accurate analysis of the time spent on various activities. In particular, distinguishing between eating TMR and eating pelleted concentrate offers deeper insights into the cows' nutritional strategies, especially the balance between mixed rations and high-energy pellet supplementation, and their influence on behaviour. Such detailed behavioural data are crucial for monitoring animal welfare and detecting deviations from normal patterns.

This technical note aims to provide researchers and technicians with a practical guide to using Solomon Coder in animal ethology. It highlights the tool's advantages, limitations, and potential applications in precision livestock farming, the analysis of nutritional behaviour, and welfare assessments.

[1] A. Orihuela, "Management of livestock behavior to improve welfare and production," *Animal*, vol. 15, suppl. 1, 100290, 2021. DOI: 10.1016/j.animal.2021.100290.

[2] T. Johansson, S. Agenäs, and M. Lindberg, "Time budgets of dairy cows in a cow-calf contact system with automatic milking," *JDS Communications*, vol. 5, no. 1, pp. 52–56, 2024. DOI: 10.3168/jdsc.2023-0401.

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Eating time of growing Simmental bulls evaluated with different approaches

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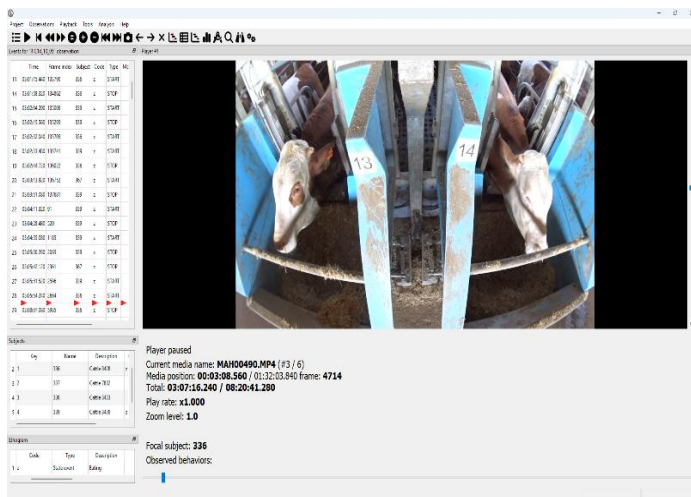


Figure 1 The main window of Boris (v.8.19.1), the software used for the visual observation of the Italian Simmental bulls to evaluate their eating behaviour.

Nowadays, in the livestock sector, it has become important to develop technologies and methods useful to monitor animals at an individual level to promote their health and welfare and to increase the sustainability of this important supply chain. Feeding behaviour in cattle is widely studied, and several systems based on different types of technologies have been developed to detect and analyse this aspect [1]. These systems are commonly used on dairy cows but are not yet so widespread in the beef cattle sector. The roughage intake control system (RIC system) is used in experimental farms to measure feed intake, but it is generally too expensive to be used at the commercial farm level. Instead, accelerometers, such as CowManager SensOor, are increasingly used as cheap and easy-to-use tools for the individual monitoring of cattle. The main goal of the current investigation is to monitor eating time using the RIC system (Hokofarm Group, Marknesse, the Netherlands) and an ear-tag accelerometer (Cow-

Manager SensOor, Agis Automatisering BV, Harmelen, the Netherlands) and to compare the outcome to visual observation. To achieve this, 23 Italian Simmental bulls were kept in pens equipped with the RIC system and fed with a total mixed ratio as detailed in previous work [2]. Animals were equipped with an ear-tag accelerometer, and video was recorded for 5 days using a camera (HDR-AS50, Sony Corp., Japan) for each pen. A trained operator has visualised a total of 345 hours and annotated the eating behaviour of bulls in three non-consecutive hours of record using Boris software (v.8.19.1) Figure 1. Comparing average data of the accelerometers and RIC system with visual observation, a significant difference ($p<0.001$) was observed for both comparisons. A Pearson correlation coefficient of 0.997 and an average difference of 0.41 min/h were observed between RIC and the camera. Instead, results for the accelerometer were 0.544 and 1.86 min/h, respectively. Furthermore, the concordance correlation coefficient was higher for cam compared to RIC (0.996) than cam compared to the accelerometer (0.544). From data collected, the RIC system seems to be a suitable tool to accurately measure the eating time of young bulls. Concerning the ear tag accelerometers, the correlation found with cam encourages the use of this technology, but further trials are needed to evaluate the main causes of overestimation in eating behaviour.

[1] Besler, B.C., Mojabi, P., Lasemiimi, Z., Murphy, J.E., Wang, Z., Baker, R., Pearson, J.M., & Fear, E.C. (2024). Scoping review of precision technologies for cattle monitoring. *Smart Agricultural Technology*, 100596. <https://doi.org/10.1016/j.atech.2024.100596>

[2] Romanzin, A., Degano, L., Vicario, D., & Spanghero, M. (2021). Feeding efficiency and behavior of young Simmental bulls selected for high growth capacity: Comparison of bulls with high vs. low residual feed intake. *Livestock Science*, 249, 104525. <https://doi.org/10.1016/j.livsci.2021.104525>

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Impact of Different Feeding Strategies on Carcass Characteristics, Meat Quality, and Organoleptic Properties of Meat from Old Sheep for *Arrosticini* Production

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In sheep farming systems, ewes that have reached the end of their average productive lifespan (estimated around 5 years) represent a significant cost to the farmer, as their productivity declines while management and feeding costs remain constant or even increase. Consequently, farmers typically slaughter these animals at this stage and sell their meat at a reduced price, leading to a decrease in economic value. This study aims to assess the effects of different feeding regimens on fattening, carcass characteristics, and meat quality in Bergamasca sheep, with a particular focus on *arrosticini* production. Specifically, the study will investigate changes in the physicochemical properties of meat, including intramuscular fat content, muscle quality, and carcass yield, in relation to the feeding strategies employed.

Twenty-six female finishing sheep (aged 4 to 6 years) were divided into three groups. The two housed groups were provided unlimited hay along with two distinct feed types: one was a sugary feed (SF), and the other a fatty feed (FF). The third group was managed under an extensive pasture system (PS). The experimental period lasted 60 days, including a 7-day adaptation phase. Growth performance and feed intake were monitored. At the end of the trial, all animals were slaughtered, and slaughter weights, 24-hour post-mortem body weights, and pH values were recorded. Samples from the longissimus dorsi were analyzed for chemical and physical properties, including the fatty acid profile and meat flavor.

A statistically significant difference was observed between the weight of the groups ($P < 0.05$). Specifically, the SF group (75.53 kg) exhibited the highest weight, followed by the FF group (74.28 kg) and the PS group (70.75 kg). Total protein percentage in the meat were similar across all three groups: 21.12% for the FF group, 20.91% for the SF group, and 20.55% for the PS group ($P > 0.05$). Also lipids content of the meat showed no differences between the groups: 4.42% for the FF group, 3.96% for the SF group, and 4.69% for the PS group ($P > 0.05$). The average values for saturated fatty acids showed no significant differences among the three groups, the same values was registered for monounsaturated fatty acids. For polyunsaturated fatty acids, the average values were 0.12% for the FF group, 0.10% for the SF group, and 0.13% for the PS group, with a p-value of 0.5. Notably, the pasture group showed a higher value only for linoleic acid ($P < 0.05$).

Overall, the results indicate that while there are some differences in fatty acid composition between feeding regimens, the majority of these differences are not statistically significant. This suggests that the type of feeding regimen, whether based on sugary or fatty feeds or extensive pasture systems, does not lead to substantial changes in the overall fatty acid profile of the meat. Consequently, these feeding strategies may not have a strong impact on the nutritional composition of the meat but the feeding strategy is able to have sheep with higher weight for *arrosticini* production, which could result in a greater economic impact on farm productivity.

Liquid Chromatography-High Resolution Mass Spectrometry Analysis of Milk as a Non-Invasive Matrix for Assessing Dairy Cows' Exposure to Perfluoro-Alkyl Substances in Italy

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Bovine milk is a vital source of nutrition, particularly for infants [1]. However, its contamination with perfluoroalkyl substances (PFASs), which are persistent pollutants with known toxicological effects, raises concerns about food safety and animal health [2]. This study analyzed the distribution of 14 PFASs in raw milk from 23 dairy cows (10 primiparous, 13 multiparous) reared in Northern Italy within a geographical area without identifiable PFASs point sources. The objective was to examine PFASs concentrations in different milk fractions (i.e. whole, skimmed milk, and cream) to identify potential differences in their accumulation and evaluate factors influencing their presence.

Milk samples were prepared by separating cream and skim fractions via centrifugation. PFASs extraction was achieved at first by using acetonitrile for protein precipitation and compound extraction, followed by solid-phase extraction with dedicated cartridges. Identification and quantification of the 14 PFASs was performed by using Liquid Chromatography coupled with High-Resolution Mass Spectrometry (LC-HRMS). The analytical method demonstrated high sensitivity and effective quality controls ensured reliable results.

All milk samples contained at least one PFASs. The most abundant compound was perfluorobutanoic acid (PFBA), with average concentrations of 317.81 ± 785.40 pg/g in skim milk, 95.73 ± 137.80 pg/g in cream, and 255.61 ± 551.30 pg/g in whole milk. Perfluorooctane sulfonate (PFOS) was significantly enriched in cream (148.30 ± 152.30 pg/g; $p < 0.001$) compared to skim milk (8.53 ± 22.50 pg/g) and whole milk (21.64 ± 56.50 pg/g), highlighting a potential affinity for lipid components. Perfluorooctanoic acid (PFOA) was also detected, albeit at lower concentrations (mean: 61.43 ± 207.40 pg/g in skim milk, 31.04 ± 52.40 pg/g in cream, 11.54 ± 27.00 pg/g in whole milk). Multiparous cows showed consistently higher PFAS concentrations across all milk fractions compared to primiparous cows, with PFBA and PFOS detected in up to 100% of samples in certain fractions.

The results underscore the widespread presence of PFASs in bovine milk, even in areas with no direct contamination sources. The enrichment of PFOS in cream highlights the potential risks associated with high-fat dairy products. These findings emphasize the need for further research into the mechanisms of PFASs excretion in cattle and their implications for food safety and animal health. Milk fat could serve as a practical biomarker for monitoring environmental contamination in dairy herds.

[1] H.M. Hammon, W. Liermann, D. Fieten, and C. Koch, "Review: Importance of colostrum supply and milk feeding intensity on gastrointestinal and systemic development in calves", *Animal*, vol. 14, pp. s133–s143, 2020.

[2] D. Schrenk, M. Bignami, L. Bodin, J.K. Chipman, J. del Mazo, B. Grasl-Kraupp, C. Hogstrand, L. Hoogenboom, Leblanc, J.C. Leblanc, Nebbia, C.S.; et al., "Risk to Human Health Related to the Presence of Perfluoroalkyl Substances in Food", *EFSA J.*, vol. 18, pp. e06223, 2020.

[3] J. Krippner, S. Falk, H. Brunn, S. Georgii, Schubert, S. Schubert, and T. Stahl, "Accumulation Potentials of Perfluoroalkyl Carboxylic Acids (PFCAs) and Perfluoroalkyl Sulfonic Acids (PFSA) in Maize (*Zea mays*)", *J. Agric. Food Chem.*, vol. 63, pp. 3646–3653, 2015.

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Advancing Veterinary Medicine with LC-MS/MS: Applications in Pharmacology, Toxicology, and Precision Diagnostics

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In an era of rapid scientific and technological advancement, mass spectrometry has also become a pivotal tool in veterinary medicine, enabling high-precision analysis for diagnostics, pharmacokinetics, and toxicology [1,2]. Among the most powerful techniques, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) offers exceptional sensitivity and specificity, facilitating the detection of drugs, contaminants, and endogenous molecules at trace levels. Its application enhances clinical diagnostics and expands research horizons, contributing to both animal, human and environmental health within a One Health framework.

Our research focuses on the development and validation of LC-MS/MS-based analytical methods to support pharmacology, toxicology, and internal medicine research. These methods comply with European Medicines Agency (EMA) guidelines [3], ensuring their reliability and applicability in various veterinary fields.

Their applications include pharmacokinetic studies of drugs, mainly antibiotics and anaesthetics. A key area of our research is the development of a therapeutic drug monitoring (TDM) program for antibiotics in foals and dogs, aimed at optimizing dosing strategies and containing antimicrobial resistance.

Our work also addresses the critical issue of environmental contaminants, particularly perfluoroalkyl substances (PFAS). Due to their persistence and endocrine-disrupting properties, PFAS pose a significant threat to both animal and human health. Leveraging robust analytical methods, we measure PFAS concentrations in environmental matrices such as freshwater, seawater, and aquatic animals, as well as in biological fluids, such as plasma and milk, of human and animal origin. Investigating PFAS in dogs and cats is of particular relevance, not only to assess potential links between exposure and endocrine disorders, including cancer and diabetes, but also as these species serve as sentinels for indoor pollution, providing insights into human exposure risks.

Additionally, we apply LC-MS/MS to quantify endogenous compounds in biological fluids, including serum, plasma, and urine. These include purines (xanthine, uric acid, and allantoin) and vitamins (such as vitamin D, and others in progress) [4] across different species. This research aims to improve disease diagnostics and deepen our understanding of metabolic pathways in animals.

The integration of LC-MS/MS into veterinary research advances precision medicine in animal care, while reinforcing the interconnectedness of environmental, animal, and human health. This interdisciplinary approach underscores the critical role of mass spectrometry in modern veterinary science, paving the way for innovative diagnostic and therapeutic strategies with broad implications.

1. Thomas, S.N.; French, D.; Jannetto, P.J.; Rappold, B.A.; Clarke, W.A. Liquid Chromatography–Tandem Mass Spectrometry for Clinical Diagnostics. *Nat. Rev. Methods Primer* **2022**, *2*, 1–14, doi:10.1038/s43586-022-00175-x.
2. Rappold, B.A. Review of the Use of Liquid Chromatography-Tandem Mass Spectrometry in Clinical Laboratories: Part II-Operations. *Ann. Lab. Med.* **2022**, *42*, 531–557, doi:10.3343/alm.2022.42.5.531.
3. EMA ICH M10 on Bioanalytical Method Validation - Scientific Guideline Available online: <https://www.ema.europa.eu/en/ich-m10-bioanalytical-method-validation-scientific-guideline> (accessed on 24 July 2023).
4. Bardhi, A.; Vecchiato, C.G.; Sabeti, M.C.; Tardo, A.M.; Vasylyeva, K.; Biagi, G.; Pietra, M.; Barbarossa, A. A Novel UHPLC–MS/MS Method for the Measurement of 25-Hydroxyvitamin D3 in Canine Serum and Its Application to Healthy Dogs. *Animals* **2024**, *14*, 62, doi:10.3390/ani14010062.

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Salinity-Driven Alterations in Tetracycline Exposed *Mytilus galloprovincialis*: A Metabolomics Perspective

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Over the past few decades, mussels have increasingly struggled with environmental changes driven by climate change. Among the key abiotic factors influencing their survival and adaptability, salinity fluctuations stand out as of primary importance [1]. Moreover, mussels filter large volumes of water and can bioaccumulate pollutants, thereby facilitating the monitoring of their exposure to a variety of chemical contaminants that may cause shifts in the metabolism of the non-target organism [2]. The ability of these organisms to quickly respond to changes in their environment is useful for molecular profiling aimed at identifying specific metabolomic profiles in response to exogenous factors. Tetracyclines (TCs) are considered one of the most widely used antibiotics class, thanks to its broad spectrum of activity, high quality, and affordability. These antibiotics play a crucial role in both human and animal medicine, helping treat infections and serving as feed additives in livestock, poultry, and aquaculture [4]. In Europe, approximately 2000 tons of TCs are consumed annually in veterinary medicine, making up about 60% of total antibiotic use [4].

Nowadays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful, high-throughput tool that provides a comprehensive understanding of the metabolic state of biological systems. Metabolomics has gained prominence in both food research and ecotoxicology, serving to monitor mussels' freshness and assess the impacts of environmental changes.

In this study, we aimed to evaluate the effects of TC exposure on *M. galloprovincialis* and examine how salinity fluctuations (20, 30, and 40) influence the organism's metabolism. Whole homogenized mussel tissue samples were extracted using a modified two-step biphasic extraction protocol. The supernatant containing polar metabolites was then analyzed for metabolomics in both positive and negative polarity. The analysis identified approximately 300 metabolites using the METLIN database. Principal Component Analysis (PCA) revealed no significant metabolic shifts in mussels exposed to TC compared to control individuals across different salinities. However, when examining the effects of salinity independently of TC exposure, individuals subjected to a salinity of 20 exhibited a distinct metabolic shift. Specifically, mussels exposed to salinity 20 showed a downregulation of phosphatidylinositols. Since negatively charged phospholipids are known to bind excess cations under higher salinity conditions, this suggests an adaptation of lipid metabolism in response to chronic exposure to environmental stressors. These findings highlight the importance of studying the impact of external factors on mussel metabolism and emphasize the need for further research to better characterize the metabolome of this organism.

[1] M. Mohsenet et al, *Sci Total Environ*, vol. 928, 2024.

[2] P. Cocci et al, *Biomarkers*, vol. 22, 2017.

[3] J. Antos et al, *Chemosphere*, vol. 353, 2024.

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¹H NMR for comparative metabolic analysis of whey and WPC-80

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Metabolites, defined as organic compounds with a low molecular weight (< 1 kDa), serve as intermediates and end products of cellular metabolism. Analysing these compounds provides insights into the quality of food products, emphasizing their inherent nutritional value and impacts on health [1]. The analysis of metabolite profiles in dairy products through proton nuclear magnetic resonance (¹H NMR) spectroscopy offers valuable insights into their nutritional composition, potential health benefits, functional and technological properties. This approach may result relevant also for whey, and whey protein concentrate (WPC). Whey is a major by-product of dairy sector, generated during milk processing into cheese and yoghurt. While raw whey is often used in animal feed or biogas production, it is also refined into products like WPC-80. WPC-80 is a premium protein product obtained through filtration and drying, concentrating whey proteins to 80% by weight while reducing water, lactose and fat content, making it a highly nutritious protein source, particularly rich in essential amino acids [2]. The present study aimed at exploring the use of ¹H NMR spectroscopy to i) characterize metabolic profiles of whey and WPC-80 and to ii) compare metabolite composition of whey and WPC-80.

A total of 5 whey and 4 WPC-80 samples were analysed through ¹H NMR spectroscopy. A Mann-Whitney U test was used to identify metabolites differing significantly between whey and WPC-80. Principal component analysis (PCA) was performed to assess group discrimination, and Partial Least Squares Discriminant Analysis (PLS-DA) identified key metabolites responsible for distinguishing whey and WPC-80.

Whey and WPC-80 showed notable metabolic differences (Fig 1). Levels of histidine, formate, glucose + glucose-6-phosphate, acetate and choline were significantly greater in WPC-80 if compared to whey (P<0.05). Conversely, dimethyl sulfone, succinate, orotate, fumarate and lactose levels were greater in whey if compared to WPC-80 (P<0.05). Among the metabolites present in both groups, lactose, histidine, and formate had the highest Variable Importance in Projection scores. Additionally, several metabolites, such as hippurate, valine, lactate + threonine, and uracil, were detected in whey but were absent in WPC-80. These differences may be attributed to the technological treatments adopted for WPC-80 production, such as the filtration process, as hippurate and valine are commonly found also in milk [4].

By identifying key nutrients, the findings of the present study can support the development of novel and functional dairy products, which may be of interest for both consumers and producers.

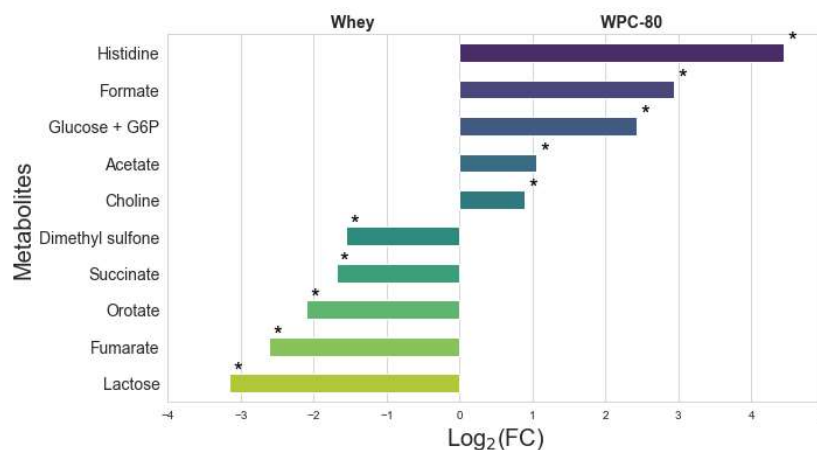


Fig 1 Logarithmic fold change [Log₂(FC)] of the area under the peaks of the identified metabolites. *P < 0.05.

[1] G. Meoni, L. Tenori, and C. Luchinat. "Nuclear magnetic resonance-based metabolomic comparison of breast milk and organic and traditional formula milk brands for infants and toddlers". *OMICS*, vol. 24, pp. 424–436, 2020.

[2] M. Falkowski, Maciejczyk M., Koprowicz T., Mikołuc B., Milewska A., Zalewska A., Car H. "Whey protein concentrate WPC-80 improves antioxidant defense systems in the salivary glands of 14-month Wistar rats". *Nutrients*, vol. 10, pp.782, 2018.

[3] G. Niero, G. Meoni, L. Tenori, C. Luchinat, G. Visentin, S. Callegaro, E. Visentin, M. Cassandro, M. De Marchi, and M. Penasa. "Grazing affects metabolic pattern of individual cow milk". *J. Dairy Sci.*, vol. 105, pp.9702–9712, 2022.

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Development of portable NIR calibration models for microplastic detection and quantification in corn silage

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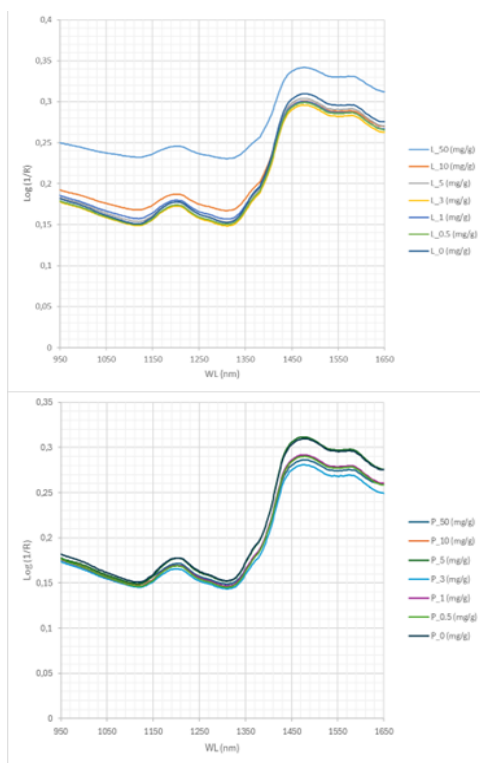


Figure 1 - Portable NIR spectra at different concentration of LDPE (L) and PS (P) microplastics in corn silage, prior to pre-processing

(SEC=4.62 mg/g DM, SEP=5.22 mg/g DM), and the MAE were very low (1.9×10^{-6} mg/g DM and 0.3 mg/g DM, respectively), though calibration residuals showed a non-normal distribution. PS also showed a good fitting calibration ($R^2=0.92$, 12 factors) but slightly lower prediction performance ($R^2=0.85$), with a higher SEP (6.3 mg/g DM) compared to SEC (4.5 mg/g). However, the MAEs remained low (2.7×10^{-7} mg/g DM in calibration and 0.40 mg/g DM in validation) but the residual distribution was non-normal in the validation phase. The model for the prediction of the contamination by PS+LDPE MPs showed the weakest performance, with a fair calibration fit ($R^2=0.89$, 14 factors) but a lower prediction fit ($R^2=0.75$). Both the standard errors (SEC=5.7 mg/g DM and SEP=8.9 mg/g DM) and MAE ($5. \times 10^{-7}$ mg/g DM in calibration and 1.5 mg/g DM in validation) were higher than those observed for the models developed per single MP type. The three models showed a good PP, always higher than 3.0.

In conclusion, portable NIRS effectively detect MP contamination in animal feed, with the PLS+PCA model showing the best results, particularly for LDPE. The findings underscore the possibility of analysing individual MPs (PS and LDPE), as this approach yields better predictive performance compared to combined models (PS+LDPE) and the portable NIRS can be a promising tool for the real-time monitoring directly MPs in corn silage without extraction.

- [1] N. Beriot, J. Peek, R. Zornoza, V. Geissen, and E. H. Lwanga. "Low density-microplastics detected in sheep faeces and soil: A case study from the intensive vegetable farming in Southeast Spain". *Science of the Total Environment*, 2021, 755, 142653. <https://doi.org/10.1016/j.scitotenv.2020.142653>
- [2] I. van der Veen, L. M. van Mourik, M. J. M. van Velzen, Q. R. Groenewoud, and H. A. Leslie. "Plastic particles in livestock feed, milk, meat and blood". 2022, https://www.plasticsoupfoundation.org/wp-content/uploads/2022/07/Livestock-mps-study-KEYmessages_final_2022-07-05.pdf
- [3] P. Berzaghi., and R. Riovanto. "Near infrared spectroscopy in animal science production: principles and applications". *Italian Journal of Animal Science*, 2009, 8(sup3), 39–62. <https://doi.org/10.4081/ijas.2009.s3.39>

Microplastics Measurements in Cow Milk: Extraction and Characterization Methodologies

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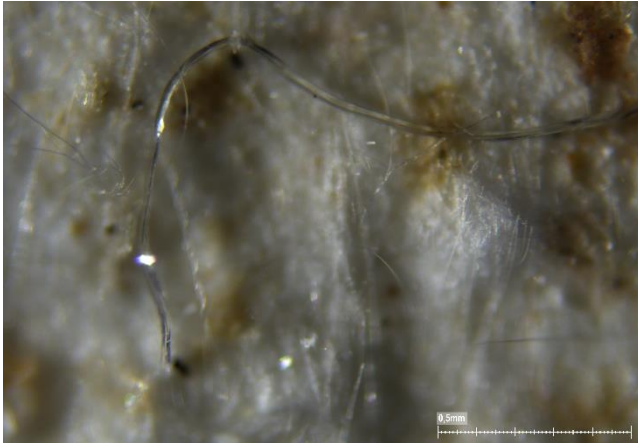


Figure 1 – Synthetic fibre on filter from UHT packaged milk.

Microplastic (MP) pollution is a growing concern that affects habitats and species, contaminating all matrices. Additionally, plastic additives and chemical pollutants linked to MPs may have toxic effects on species.

Currently, only few studies investigating the presence of MPs in farmed animals and their products, and research about the transmission of MPs from livestock products to humans is just at the early stage. Different studies have shown that MPs can accumulate in the digestive systems of some animals, causing inflammation, stress, and possible disruptions in nutrient absorption. MPs have been found in milk, meat, and other animal-derived products, raising food safety concerns. Therefore, scientists emphasize the need for further research to assess the risks.

MP contamination in milk were detected in raw and processed milk, likely originating from contaminated feed, water, and plastic packaging. Livestock can ingest

MPs through contaminated feed and water leading to potential health implications for animals and human consumers. Additionally, processing equipment and plastic containers may contribute to further contamination.

The aim of this study is to find a valid method for MPs separation and characterisation in milk, following the product from milking to packaging to mitigate the impact of MPs in livestock farming.

Milk samples were collected from farm to supermarket, observing different problematics depending on the fat presence, especially in raw milk. Major problematics regarded the milk filtration, due to the presence of fats. Heating the milk at different temperatures and the use of KOH 10% can partially solve these problems, helping in milk filtration. Different filters were tested too, highlighting the need of high porosity for raw milk. Both microscopic and spectroscopic techniques can be useful to count and characterize MPs on filter. The use of UV light can help in MP identification, especially for small and clear particles. Blank samples and the filtration of chemicals and distilled water used is essential to avoid MP contamination during all step, from sampling to microscopic and spectroscopic analysis.

Further studies are necessary to assess the full impact of MPs in milk and potential risks to consumers. Addressing MP contamination in livestock farming is crucial for protecting both animal and human health. Collaborative efforts between policymakers, researchers, and farmers are necessary for developing sustainable solutions, and reduce plastic in agriculture and food production.

Enhancing microplastic detection directly in feeds using ghost NIR spectra

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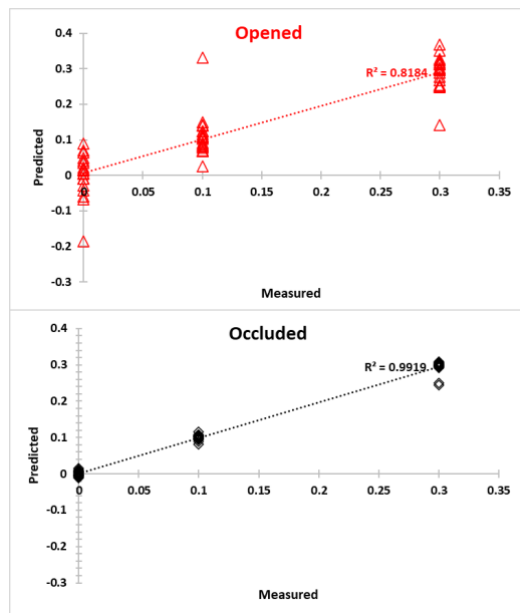


Figure 1. Performances of the NIRS evaluation related to the 3 MP concentrations (0.0, 0.1, and 0.3%).

Near InfraRed Spectroscopy (NIRS) is commonly employed in agriculture for bromatological and rapid analyses. This study introduces a novel approach discovered through an unexpected error, which uncovered a hidden potential to enhance the NIR spectroscopic estimation of microplastic (MPs) content in two feeds of vastly different compositions. The method leverages "occluded" radiation, referring to the radiation detected by the instrument even when the shutter is closed. This observation was successfully replicated on additional samples using the same equipment, prompting us to share these results. In this study, we present a comparative analysis of two spectral datasets (Opened and Occluded shutter) consisting of 60 *spectra* - half Total Mixed Ration, and half Hay - across three different levels of green low-density polyethylene MPs (0.0, 0.1, and 0.3%).

The samples were analyzed in reflectance mode using a benchmark IdentiCheck™ FT-NIR-IR system (PE, Perkin-Elmer, Beaconsfield, England), covering the 714-3333 nm range with 2751 absorbance points, as described in [1]. Each absorbance *spectrum* was the average of 26 scans, with each sample being mixed and replicated 10 times. Each sample was first measured with the visible laser beam, followed immediately by a measurement with the shutter occluded to prevent direct reflection. The sample was then randomly repositioned, and this measurement procedure was repeated. The absorbance *spectra* were imported into GRAMS/AI™ 7.02 software (Win-ISI III, Infrasoft International, Port Matilda, PA, USA) and

processed using the modified Partial Least Squares method, both as raw data, after standardization and first or second derivative transformations. Model stability was evaluated through cross-validation, which allowed for the removal of outliers exceeding the critical values of 'T-Student' = 2.5 and 'Global H' = 10. The Standard Error of Cross Validation (SECV) and the Ratio of Performance to Deviation (RPD) were used as performance indicators.

The absorbance value (Log 1/R) obtained from the open shutter was 0.211 ± 0.398 (N=165060). In contrast, the occluded absorbance signal, diffused from the closed shutter, was significantly lower at 0.014 ± 0.384 - 93% lower than the open signal, though still greater than zero ($P < 0.0001$). The correlograms comparing concentration vs NIR *spectra* showed distinct differences between open and occluded shutter: low and constant correlation in the open mode, and high and highly variable in the occluded mode. Accuracy (SECV) were 0.2% in closed mode vs 0.6% in open mode, with RPD values of 5.5 and 2.2, respectively. For comparison, Corradini et al. [2] for LDPE reported an accuracy of 0.8% for soil.

The phenomenon of detecting occluded/ghost radiation is likely the result of a combination of the optical properties of the sample, the sensitivity of the FT-NIR-IR instrument, and the specific way light interacts with the feed materials and microplastics. The closed shutter may allow some radiation to still be detected due to scattering, internal reflections, and the material properties of the feed. This results in a weak, but measurable, signal that could be used to enhance the detection of microplastics in feeds, even when they are not immediately visible in the sample. The ability to replicate this observation across different samples further suggests that this phenomenon is a consistent and reliable feature that could improve NIRS-based analysis.

[1] S. Tassone, G. Masoero, and P.G. Peiretti, "Vibrational spectroscopy to predict *in vitro* digestibility and the maturity index of different forage crops during the growing cycle and after freeze- or oven-drying treatment," *Anim. Feed Sci. and Tech.*, vol. 194, pp. 12-15, 2014.

[2] F. Corradini, H. Bartholomeus, E.H. Lwanga, H. Gertsen, H., and V. Geissen, "Predicting soil microplastic concentration using vis-NIR spectroscopy," *Sci. Tot. Env.*, vol. 650, pp.922-932, 2019.

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EVALUATION OF LEARNING CURVE FOR POINT-OF-CARE ULTRASOUND PROTOCOL IN EQUINE SPECIES: PRELIMINARY STUDY

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Point-of-care ultrasonography (POCUS) is a valuable tool that enhances physical examinations and patient-side procedures, with growing evidence showing its ability to modify diagnoses, guide further testing, and influence medical management [1]. However, as a highly operator-dependent technique, its safe and effective use requires multiple steps, with image acquisition often considered the most critical [2]. In equine medicine, studies on POCUS are limited and provide minimal guidance on the skill level or learning process needed to acquire images [3].

The present study evaluated the learning curve of a foal POCUS protocol by comparing three operators with different skill levels (expert: A; intermediate: B; novice: C).

The protocol included abdominal, thoracic and cardiac views [4] performed on 10 foals of different breeds aged 4-73 days. Cineloops image sonograms of key organs/structures were captured from each view. For each sonogram successful image acquisition (yes/no), the time required to acquire the image, and the image maintenance time were recorded. Data were expressed as median/percentile for continuous variables and frequency/percentage for categorical variables. ANOVA test was performed to compare the time required to acquire the image between the operators, Kruskal-Wallis was used to compare the image maintenance time between operators, and a Cohen’s Kappa test was used to compare successful image acquisition between operators. A p-value < 0.05 was considered significant.

The prevalence of successfully collected images was the same for all 3 operators (93.8%). There was no difference in the time of operators A and B to successfully acquire images, while operator C was able to acquire images more quickly over time (p=0.002). Although a trend was noted, there was no statistically significant difference in image maintenance time between operators (p=0.056). However, a statistically significant difference was observed for operator A vs. B (p = 0.033) and A vs C (p = 0.015) to maintain the right parasternal long-axis view of the left ventricular outflow tract. Results should be interpreted with caution as the small sample size may have created a type II error.

In conclusion, the prevalence of successful image acquisition might not depend on the operator’s level of experience, but the operators’ skill influences the acquisition time. Given the novice became more proficient over time, a steep learning curve for POCUS image acquisition seems to exist in foals.

[1] E. Kaffas, “Critical advances for democratizing ultrasound diagnostics in human and veterinary medicine”, *Annu. Rev. Biomed. Eng.*, vol. 26, pp. 49-65, 2024.

[2] Wagner MS, Garcia K, Martin DS (2014) Point-of-care ultrasound in aerospace medicine: known and potential applications. *Aviat Space Environ Med* 85(7):730–739

[3] C. Eberhardt, “Focused cardiac ultrasound examination in the emergency and critical care horse: Training for non-specialist veterinarians and evaluation of proficiency”, *J. Vet. Intern. Med.*, vol. 36, pp. 1471-80, 2022.

[4] Kidd JA et al. (2022). “Atlas of equine ultrasonography”. 2nd edn. Wiley-Blackwell Ltd.

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Reliability of a digital stethoscope associated with phonocardiography for cardiac auscultation in dogs

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Figure 1 – Example of a recording with ECG and PCG and the technique used to record the various tracks with medium-large dogs.

Auscultation is essential for the early diagnosis of cardiac diseases, yet traditional stethoscopes (CS) face limitations like subjectivity and the limitation of human hearing. To address these, digital stethoscopes (DS) have been developed to enhance sound quality, allow recording, and enable sharing. Numerous studies have compared the effectiveness of DS with CS [1, 2]. In particular recently, the “Eko DUO ECG + Digital Stethoscope” has garnered interest in veterinary medicine [3, 4]. This study aimed to compare auscultatory findings from a CS and the DS “Eko DUO ECG + Digital Stethoscope” with echocardiographic diagnoses. Secondary objectives included analyzing inter-operator variability and assessing an AI function. This observational, prospective study involved dogs with heart diseases and healthy controls. Each underwent conventional auscultation, echocardiography, and DS recording. An expert operator blindly analyzed recordings, assessing agreement with echocardiographic results using Cohen’s kappa (k) test. The same analysis was performed on auscultatory findings via CS. In a subgroup of 35 dogs, agreement regarding the presence of murmur and its characterisation was assessed between three operators with different experience using the Fleiss k-test. In another subgroup of 23 dogs, agreement between CS, DS, and AI-based murmur identification was evaluated with Cohen’s k. A total of 165 dogs were included,

with all audio recordings deemed interpretable and 59% rated of excellent quality. Both DS and CS showed substantial agreement with echocardiographic findings in identifying cardiac murmurs ($k=0.72$ for DS; $k=0.64$ for CS), with DS demonstrating greater sensitivity for murmur detection (86% vs 80%). Inter-operator agreement was substantial in identifying heart murmurs ($k=0.72$) and murmur intensity ($k=0.71$), but only slight agreement for murmur duration ($k=0.36$). Finally, the AI-based murmur identification aligned well with both CS and DS results, showing substantial agreement ($k=0.74$). The present study suggests a good accuracy of the device in the identification of heart murmurs in dogs, and a good agreement between different operators. Additionally, the integrated AI function shows potential as a supportive tool for murmur identification. These features highlight its possible value for canine cardiologic screening, telehealth, and teaching purposes.

[1] Tavel ME. Cardiac auscultation: a glorious past and it does have a future! *Circulation*. 113:1255-9, 2006.

[2] Höglund K, Ahlstrom CH, Häggström J, Ask PN, Hult PH, Kvart C. Time-frequency and complexity analyses for differentiation of physiologic murmurs from heart murmurs caused by aortic stenosis in Boxers. *Am J Vet Res*. 68:962-9, 2007.

[3] Vezzosi T, Alibrandi L, Grosso G, Tognetti R. Evaluation of a new smartphone-based digital stethoscope featuring phonocardiography and electrocardiography in dogs and cats. *Vet J*. 295:105987, 2023.

[4] Saponaro V, Mey C, Vonfeld I, Chamagne A, Alvarado MP, Cadore JL, Chetboul V, Desquilbet L. Systolic third sound associated with systolic anterior motion of the mitral valve in cats with obstructive hypertrophic cardiomyopathy. *J Vet Intern Med*. 37:1679-1684, 2023.

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Applicability of Two-Dimensional Shear Wave Elastography for assessing hepatic stiffness in dogs with right-sided congestive heart failure

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Two-Dimensional Shear Wave Elastography (2D-SWE) is a novel, non-invasive ultrasound technique used to assess the elasticity of an organ's parenchyma. In human medicine, ultrasound elastography is commonly employed to evaluate hepatic stiffness, which can potentially indicate pathological liver conditions and has been shown to correlate with histopathological findings. [1] This method is non-invasive, cost-effective, and easy to apply. [2]

In human medicine, there are studies evaluating the use of 2D-SWE in cases of hepatic congestion secondary to right-sided congestive heart failure (R-CHF), demonstrating a significant correlation between hepatic parenchymal stiffness and the severity and prognosis of cardiac disease. [3,4] Since there are no similar studies in veterinary medicine, the aim of our prospective study was to evaluate the applicability of 2D-SWE in dogs with R-CHF, assessing the possible associations between hepatic stiffness values and ultrasound vascular markers of R-CHF.

The study included 50 dogs, comprising 38 healthy patients and 12 with R-CHF, conducted at the Veterinary Teaching Hospital "M. Modenato" of the Department of Veterinary Sciences at the University of Pisa. Each dog underwent 2D-SWE elastosonographic examination, and the pathological patients also underwent B-mode ultrasound evaluation of the dimension of right and left hepatic veins and caudal vena cava (caudal vena cava maximal size indexed to aortic dimension and caudal vena cava collapsibility index). Pathological patients exhibited significantly increased median values of hepatic stiffness compared to healthy subjects (1.40 vs. 1.32 m/s, $p=0.002$; 6 vs. 5 kPa, $p=0.004$). No correlations were found between hepatic stiffness values and the vascular parameters under consideration. Two-dimensional Shear Wave Elastography appears promising for potential application in dogs with R-CHF.

[1] Sigrist RMS, Liao J, Kaffas AE, Chammas MC, Willmann JK. Ultrasound elastography: review of techniques and clinical applications. *Theranostics* 2017;7:1303-29.

[2] Dong Y, Sirli R, Ferraioli G, et al. Shear wave elastography of the liver - review on normal values. *Z Gastroenterol* 2017;55:153-66.

[3] Rako ZA, Yogeswaran A, Yildiz S, Weidemann P, Zedler D, da Rocha BB, Kryvenko V, Schäfer S, Ghofrani HA, Seeger W, Kremer NC, Tello K. Liver stiffness is associated with right heart dysfunction, cardiohepatic syndrome, and prognosis in pulmonary hypertension. *J Heart Lung Transplant*. 2024 Jul;43(7):1105-1115.

[4] Dhillon JK, Fong MW, Fong TL. Use of liver stiffness measurements in acute decompensated heart failure: new applications of a non-invasive technique. *ESC Heart Fail*. 2022 Oct;9(5):2800-2807.

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Smart textile biotechnology for ECG monitoring in minipigs: A validation study

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Minipigs are increasingly used as animal models in biomedical research [1,2], particularly in cardiology [3]. Pre-clinical research requires electrocardiography (ECG) to assess in animal models health status [3]. However, no comparison has been made between different ECG monitoring systems in minipigs. Smart textiles are seen as promising biotechnology [4]. Our study aimed to investigate whether measurements from smart textile biotechnology (Smartex Srl, Navacchio, Italy) were consistent with measurements from a telemetric ECG system used in veterinary medicine (Televet®, Engel Engineering GmbH, Heusenstamm, Germany).

This study was conducted with authorisation n 812/2021-PR dated 10/29/21 by the OPBA of CNR of Pisa. The study involved 10 minipigs (7 females and 3 males) that underwent anaesthesia with azaperone (2 mg/kg IM) and the association of zolazepam-tiletamine (2 mg/kg IM) [5,6]. Ten-minute ECGs were recorded on each animal using Televet® (500 Hz) and Smartex (250 Hz). All the ECGs were inspected, corrected, synchronised and then segmented into time windows: four two-minute windows were used to extrapolate mean heart rate (MeanHR) and Heart Rate Variability (HRV) parameters of time domain (MeanRR, SDNN, RMSSD), frequency domain (LF/HF) and non-linear (SD1, SD2, SampEn) for each animal.

The raw data and the differences in the values of the parameters detected by the two monitoring systems were tested for normality (Shapiro-Wilk Test) and showed a non-normal distribution. The Spearman's rank correlation coefficient (ρ) was calculated, and the Bland-Altman plot was used to assess the agreement between the data collected by the two monitoring systems [7,8].

All detected parameters were very strongly correlated ($\rho > 0.9$, $p\text{-value} < 0.001$) between the two monitoring systems, except for SampEn, which showed a positive moderate correlation ($\rho > 0.6$, $p\text{-value} < 0.001$). The Bland-Altman plots showed a general agreement between the two monitoring systems, for all the parameters detected. This is consistent with what has already been reported in studies comparing Televet® and Smartex in sheep [9] and horses [10].

Overall, our study showed that smart textile biotechnology is reliable for assessing the mean HR and HRV parameters in minipigs. This study advances knowledge of non-invasive biomedical technologies, potentially improving the welfare of animal models.

- [1] M. Kuwahara, A. Suzuki, H. Tsutsumi, M. Tanigawa, H. Tsubone, and S. Sugano, "Power Spectral Analysis of Heart Rate Variability for Assessment of Diurnal Variation of Autonomic Nervous Activity in Miniature Swine", *Lab. Anim. Sci.*, vol. 49, pp. 202-208, 1999
- [2] K. Nahas, P. Baneux, and D. Detweiler, "Electrocardiographic Monitoring in the Göttingen Minipig", *Comp. Med.*, vol. 52, pp. 258-264, 2002
- [3] M. K. Lyhne, K. P. Debes, T. Helgogaard, A. Vegge, J. Kildegaard, U. Pedersen-Bjergaard, and L. H. Olsen, "Electrocardiography and heart rate variability in Göttingen Minipigs: Impact of diurnal variation, lead placement, repeatability and streptozotocin-induced diabetes", *J. Pharmacol. Toxicol. Methods*, vol. 118, pp. 1-12, 2022
- [4] M. Felici, M. Nardelli, A. Lanatà, M. Sgorbini, E. P. Scilingo, and P. Baragli, "Smart textiles biotechnology for electrocardiogram monitoring in horses during exercise on treadmill: Validation tests", *Equine Vet. J.*, vol. 00, pp.1-6, 2020
- [5] Y. S. Kim, M. J. Kim, S. J. Lee, J. I. Lee, M. H. Jun, C. S. Park, and M. C. Kim, "Comparison of anesthetic effects induced by tiletamine-zolazepam and azaperone plus tiletamine-zolazepam in growing pigs", *J. Vet. Clin.*, vol. 24, pp. 300-304, 2007
- [6] R. Costea, I. Ene, and R. Pavel, "Pig Sedation and Anesthesia for Medical Research", *Animals*, vol. 13, pp. 1-17, 2023
- [7] J. M. Bland and D. G. Altman, "Measuring agreement in method comparison studies", *Stat. Methods Med. Res.*, vol. 8, pp. 135-160, 1999
- [8] D. Giavarina, "Understanding Bland Altman analysis", *Biochem. Med.*, vol. 25, pp. 141-151, 2015
- [9] L. Turini, F. Bonelli, A. Lanatà, V. Vitale, I. Nocera, M. Sgorbini, and M. Mele, "Validation of a new smart textiles biotechnology for heart rate variability monitoring in sheep", *Front. Vet. Sci.*, vol. 9, pp. 1-13, 2022
- [10] P. McCrae, H. Spong, N. Golestani, A. Mahnam, Y. Bashura, and W. Pearson, "Validation of an Equine Smart Textile System for Heart Rate Variability: A Preliminary Study", *Animals (Basel)*, vol. 13, pp. 1-12, 2023

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Electrocardiographic Recording and Sharing Using Digital Devices in Equids

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Electrocardiography is considered the gold standard for the diagnosis of arrhythmias. Standard electrocardiographic (ECG) examination requires an electrocardiography machine, with cables and electrodes, and usually access to electricity; however, standard electrocardiographs are not always practical to use when ECGs are performed in the field [1,2]. In recent years, innovative smartphone-based digital devices (SBDD) have been developed allowing the recording of ECGs directly on a smartphone or a tablet, and their visualization using a dedicated app and/or website, providing the possibility to store the ECGs online and share it among veterinarians, making interprofessional consultations for remote expertise much easier. In equines, studies describing the use of SBDD mainly focus on the validation of these ECG tools comparing digital ECG tracings with those obtained using a standard electrocardiograph [2,3,4,5,6,7]. Similarly, a preliminary study verified the usefulness of a single lead smartphone-based digital stethoscope device in recording ECGs in donkeys in the field, comparing them with ECGs obtained using a traditional base-apex lead electrocardiograph [1]. The available literature showed that SBDDs have good accuracy for the assessment of heart rate and rhythm in equids [2,3,4,5,6,7]. The use of these SBDDs has great importance, since they allow veterinarians to gather and share information about patients, providing support even to less experienced colleagues and offering new opportunities to improve access to care, streamline the consultation process, and closely monitor the health of animals more easily.

[1] C. Bozzola et al., "Evaluation of a digital stethoscope for electrocardiographic recording in donkeys: preliminary results" *J Equine Vet Sci*, vol. 135, 2024.

[2] E. Alberti et al. "Evaluation of a smartphone-based electrocardiogram device accuracy in the field and in hospital conditions in horses" *Vet Rec Open*, vol. 7, 2020.

[3] T. Vezzosi et al. "Evaluation of a smartphone electrocardiograph in healthy horses: comparison with standard base-apex electrocardiography" *J Equine Vet Sci*, vol. 67, 2018.

[4] F. Bindi et al. "A new smartphone-based digital stethoscope featuring phonocardiography and electrocardiography in adult horses." *Vet Res Commun*, vol. 49, pp. 43, 2025.

[5] F. Bindi et al. "Evaluation of a smartphone electrocardiograph in healthy foals and comparison to standard base-apex electrocardiography" *Vet Res Commun* vol. 48, 2024.

[6] V. Vitale et al. "Evaluation of a new portable 1-lead digital cardiac monitor (eKuore) compared with standard base-apex electrocardiography in healthy horses" *Plos One* vol. 16, 2021.

[7] B. Welch-Huston et al. "Comparison between smartphone electrocardiography and standard three-lead base apex electrocardiography in healthy horses" *Vet Rec* vol. 187, 2020.

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Pilot study for the feasibility of deep Photoacoustic imaging in pig bladder throughout developed laser catheter consumable

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The management of bladder cancer is particularly challenging due to the difficulty in preventing the progression of early non-muscle-invasive forms, specifically carcinoma in situ [1]. These lesions are often elusive, characterized by their flat morphology and small cellular clusters, which complicates diagnosis. Traditional diagnostic methods frequently fail to identify these minute lesions, hindering effective prevention strategies. To address this issue, we are exploring photoacoustic imaging, a non-invasive and non-ionizing technique that integrates laser and ultrasound technologies [2]. Utilizing a porcine model, we have established an experimental framework that simulates human bladder dimensions. Our research focuses on the use of gold nanorods (GNRs) as imaging nano-platforms, facilitated by a suitable optical fiber designed to illuminate the bladder from within, thereby overcoming the depth penetration limitations inherent in photoacoustic imaging. The imaging was conducted using the multimodal imaging platform Vevo LAZR X (FUJIFILM Visualsonics Inc.) across two phases: *ex vivo* and *in vivo*. In the *ex vivo* phase, GNRs were injected into the bladder walls of four euthanized pigs, while the *in vivo* phase involved a similar procedure under general anaesthesia in four farm pigs, ensuring the bladder was adequately prepared for imaging. The urinary catheter was substituted with a new optical fiber. The probe, oriented transversely to the animal's spine, enabling an examination of the caudal abdomen. The bladder was situated beneath approximately 2-3 cm of adipose tissue that separated it from the peritoneum. Upon locating the bladder, a comprehensive scan was conducted, starting from the urethra to the cranial region to capture the entire structure. An in-plane technique facilitated the insertion of a syringe needle in a lateral-medial direction, allowing for the injection of 0.02 mL of gold nanoparticles at a concentration of 50 μM . To visualize the nanoparticles on the ventral surface of the bladder, three injections were administered into the bladder wall. Both *ex-vivo* and *in-vivo* tests validated the capability of the custom-designed standard fiber to activate plasmonic currents of the nanoparticles through light stimulation, achieving a signal-to-noise ratio (SNR) between 75 and 100 in *ex-vivo* samples, and between 20–40 in *in-vivo* experiments. This study established the viability of employing a photoacoustic technique for the bladder biomedical imaging through laser light catheterization capable to detect the PA signal from the GNRs injected, otherwise undetectable by external standard illumination, due to the depth of the situ correlated with the effects of light absorption and scattering.

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[1] Ferlay J et al. Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. *Eur J Cancer*. 2018 Nov;103:356-387.

[2] Kamaya et al. Photoacoustic imaging of the bladder: a pilot study. *J Ultrasound Med*. 2013 Jul;32(7):1245-50.

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Computed Tomography (CT) Scan Measurements for the Assessment of the Appropriate Tidal Volume to Apply in Mechanically Ventilated Rabbits Under General Anesthesia

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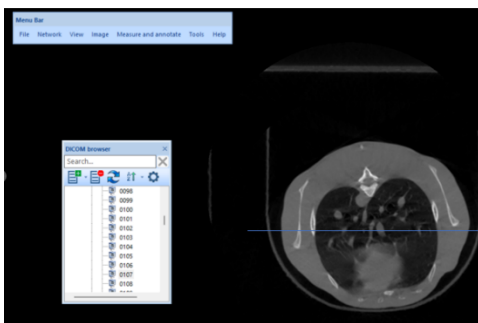


Figure 1. Representation of a slice (CT scan) selected at the level of the 6th rib of a subject included in the study. The lung was divided (blue line) into an apical and dependent portion.

The aim of this study was to evaluate hypo-aerated, normo-aerated and hyper-aerated lung areas in mechanically ventilated rabbits, by analyzing CT scans using the MicroDicom-DICOM Viewer program (version 2022.3). For this purpose, 24 rabbits (*Oryctolagus cuniculus*) were selected, subjected to preoperative CT control and subsequent dental surgery. All patients were premedicated with dexmedetomidine (80 mcg/kg), ketamine (5 mg/kg) and methadone (0.2 mg/kg), intubated and maintained with isoflurane in pure oxygen. Rabbits were mechanically ventilated in volume-controlled mode with different tidal volumes: TV = 6 ml/kg (group L), TV = 10 ml/kg (group M) and TV = 12 ml/kg (group H). The ventilatory setting was: respiratory rate (RR) = 15-18 breaths/min; inspiratory pause 25%; I:E ratio 1:2; PEEP 0 cmH₂O. After 5 minutes of adaptation to mechanical ventilation (baseline), the main cardiovascular and respiratory parameters were monitored and, in addition, an arterial blood sam-

pling and a CT scan were performed. The same procedure was performed after 20 minutes (T20). Subsequently, the intrapulmonary shunt (Fshunt), the alveolar-arterial oxygen and carbon dioxide gradient, and the indexed static and dynamic compliance (CstatIND and CdynIND, respectively) were calculated. Regarding the CT scan measurements, the slices at the level of the 2nd and 6th rib were selected, and, in each slice, two ROIs were measured, dividing the lung into an apical and a dependent portion in each study group. Lung aeration of each region was then analyzed considering hyper-aerated (-1000/-901 HUS), normo-aerated (-900/-501 HUS), hypo-aerated (-500/-101 HUS) and non-aerated (-100/+100 HUS) areas based on what was previously demonstrated in the canine species [1]. Values of $p < 0.05$ were considered statistically significant. ROI analysis demonstrated that group L showed several hypo-aerated areas both at baseline and at T20. On the other hand, in group H, all analyzed areas were normo-aerated and there were no regions of hyper-aeration. CstatIND and CdynIND were significantly higher in group H compared to the other 2 groups, both at baseline and at T20. Furthermore, in group H, at T20, Fshunt was significantly lower than baseline in the same group and compared to the other 2 groups at the same time point in the study. In conclusion, our data show that a high TV (12 mL/kg) could be ideal for volume-controlled ventilation of rabbits, similar to what has been previously demonstrated in dogs [2]. However, there are currently no studies regarding CT-measurable lung densities in rabbits.

Figure 1. Representation of a slice (CT scan) selected at the level of the 6th rib of a subject included in the study. The lung was divided (blue line) into an apical and dependent portion.

[1] De Monte V, Bufalari A, Grasso S, Ferrulli F, Crovace AM, Lacitignola L, et al. Respiratory effects of low versus high tidal volume with or without positive end-expiratory pressure in anesthetized dogs with healthy lungs. *Am J Vet Res.* (2018) 79:496–504. doi: 10.2460/ajvr.79.5.496

[2] Bumbachera S, Schramelb JP, Mosing M. Evaluation of three tidal volumes (10, 12 and 15 mL/kg) in dogs for controlled mechanical ventilation assessed by volumetric capnography: a randomized clinical trial. *Vet Anaest Analg* (2017) 44(4):775-784. doi: 10.1016/j.vaa.2016.10.007

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Validation of a Novel Technological Device for Vital Functions Monitoring in Hospitalized Dogs

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Continuous monitoring of vital parameters is extremely useful in hospitalized dogs, for assisting clinicians in patient management. The aim of this study was to validate the performance of a novel multiparametric wearable device developed for the telemonitoring of dogs.

Eligibility criteria for dog enrollment included having a balanced body shape perfectly adaptable to the device, a normal mental status, normal posture and gait, and a stable, low-risk clinical state. Measurements of heart rate (HR), body temperature (T°C), and respiratory rate (RR) were performed in eight dogs using the device Dinbeat UNO® (Dindog Tech, Spain). Additionally, it recorded an electrocardiogram (ECG) (presence/absence of arrhythmia), different positions (Ps) (standing/lying), activities (As) (rest/movement), and vocalization (Vs). The recording took a total of 230 minutes, out of them 170 in cages and 30 outsides (free movement).

Data were compared with different gold standards consisting of direct clinical procedures: body temperature measurement with a digital thermometer (SC 1091 Veterinary thermometer Flex), HR and ECG assessment using a multiparametric monitor (MINDRAY uMEC 12 VET SN V7-0A000461), and RR, Ps, As, and Vs recording via direct observation.

The correlation between continuous variables was assessed using Pearson's test (r), while categorical data (i.e., ECG, Ps, As, Vs) were evaluated using χ^2 -test's contingency tables. For categorical variables, sensitivity (Se), specificity (Sp), positive and negative predictive value (PPV and NPV) were calculated.

Overall, 248 values for the RR parameter, 347 for the HR, 227 for the T°C, and 248 for the ECG were recorded; instead, 260 evaluations were instead performed for Ps, As, and Vs. A strong correlation was observed for HR, RR, and T°C variables ($r = 0.994$, $r = 0.745$, and $r = 0.924$, respectively; $P < 0.0001$). For categorical variables, no significative differences were found between the device performance and the gold standard. Finally, Se, Sp, PPV and NPV were highest for the variables ECG, Ms, Vs (values=1.00); instead, a Se of 0.73 a Sp of 0.91, a PPV of 0.73, and a NPV of 0.91 were instead detected for Ps variable. The preliminary analysis shows satisfactory results that might allow us to hypothesize future uses for remote monitoring in uncooperative patients or those requiring minimal handling. However, further studies are needed to evaluate its performance and consistency in animals with different body shapes and in patients with severe impairment of their health status.

Assessing the health of farmed rainbow trout (*Oncorhynchus mykiss*): use of an open-source software for intestine histology

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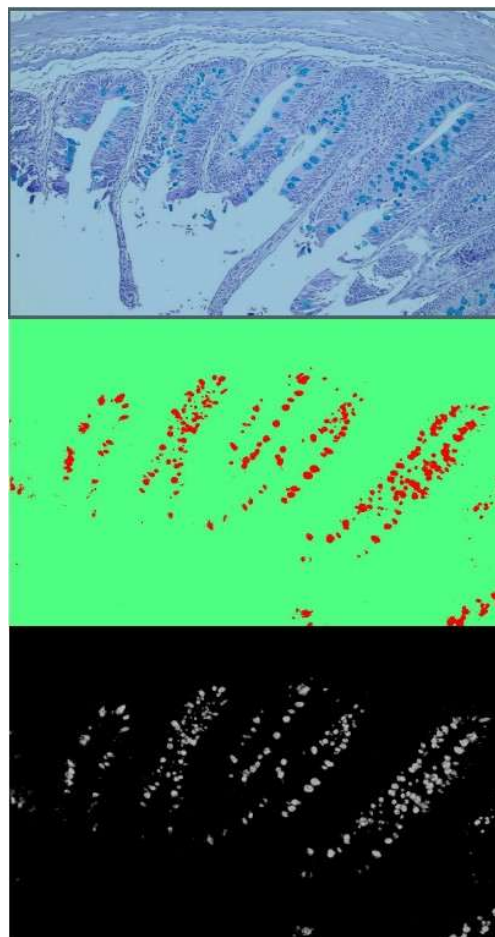


Figure 1 - Sequence of the reprocessing of the histological image with ImageJ software for the goblet cells count in the proximal intestine of rainbow trout.

Open-source software are providing opportunities and advantages to operators in several fields as fish welfare.

This study is part of a 90-days feeding trial where three diets were tested in rainbow trout (*Oncorhynchus mykiss*). By substituting guar meal protein concentrate (MYCOPRIME[®]) for 0% (CD), 5% (D5), and 15% (D15) of traditional protein sources, the diets were formulated to have a protein level of 43% and a lipid content of 25.3%.

The proximal intestines of 15 rainbow trout per diet were sampled and Haematoxylin and Eosin staining was applied to histological sections, performing a blind, semi-quantitative assessment. Every section was thoroughly examined at low (10×) and medium (20×) magnification, considering as pathological traits: inflammation (eosinophilic granular cells, goblet cell hyperplasia, and infiltration of macrophages); intraepithelial lymphocytes; steatosis in enterocytes. A score from 0 to 3, corresponding to the severity degree of the microscopical tissue structure modification, has been allocated to all the parameters evaluated.

Additionally, the histological sections were stained using Alcian Blue kit to study the goblet cells. For each sample, four random microscopical fields were chosen and 20x magnification images were taken. Consequently, the open-source program ImageJ (Fiji) was used for a quantitative assessment of the goblet cells. The algorithm was then trained to recognize Alcian blue-stained vacuoles, omitting smaller areas than 20 pixels², since these areas would have been too small to be a true vacuole (Figure 1).

For the statistical analysis, GraphPad Prism 9 was used and Kruskal–Wallis and a Dunn's multiple comparisons tests performed (p -value<0.05 significant).

The CD group had the highest histological score, while the D15 group had the lowest (p <0.001). Goblet cell hyperplasia showed significant differences between the CD group and the D15 group and even the goblet cell count difference resulted to be significant (p <0.001).

According to a recent study, the proximal intestine of healthy rainbow trout has much larger vacuoles and significantly more goblet cells [1], suggesting a potential function in gut health. The smaller number of goblet cells in the D15 group may be associated to the animals' poorer zootechnical performances when no other pathological alterations are present.

The quantitative histological assessment by means of the software permitted a more comparable and clear differentiation of the effects of the diets.

In conclusion, guar meal protein concentrate was shown to be suitable for use in rainbow trout feed formulation during the growing period, although the effect on the intestinal mucosa should be further developed.

[1] N. Verdile, R. Pasquariello, M. Scolari, G. Scirè, T.A. Brevini, and F. Gandolfi, "A detailed study of rainbow trout (*Oncorhynchus mykiss*) intestine revealed that digestive and absorptive functions are not linearly distributed along its length. *Animals*, 10, 745, 2020.

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Measurement of intestinal morphology, growth rate, and immunity response in Nile tilapia (*Oreochromis niloticus*) fed with Slab51 probiotic mixture

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The Nile tilapia (*Oreochromis niloticus*) a widely farmed fish species, ranking second only to carps¹. Its success in aquaculture is attributed to the adaptability to environmental variations, short rearing cycle, high disease resistance, and low dependency on artificial feeds². Feed quality is crucial in the growth, survival, and production efficiency of Nile tilapia, directly impacting water quality and nutrient utilization. Among nutritional strategies, probiotics have gained attention for their ability to enhance immune responses, improve nutrient absorption, and modulate intestinal structure and microbiota^{3,4}. Histological measurement of gut morphology (e.g. villus width, length or crypt depth) is considered a reliable indicator of gut functionality, particularly in relation to the absorptive surface and subsequent conversion of nutrients.

This study investigated the effects of a multispecies probiotic formulation (Slab51) on the intestinal morphology, growth performance, immunity, and feed utilization of Nile tilapia. A 60-day feeding trial was conducted using fingerlings (7.55 ± 1.25 g) randomly distributed into three replicate aquaria for each treatment: control, Slab51 in feed and Slab51 in water. At the end of the trial, growth parameters (weight gain, %WG, specific growth rate [SGR]), feed utilization indices (protein efficiency ratio [PER], feed conversion ratio [FCR]), immune activity (respiratory burst test on spleen macrophages), and intestinal morphology were evaluated.

The most striking findings were observed in intestinal morphology. Fish fed Slab51-supplemented diets exhibited significant increases in villus length, width, and area ($p < 0.01$), marked enhancements in mucosal fold height, lamina propria thickness, and the villus width-to-length ratio. Additionally, the number of goblet cells increased significantly ($p < 0.05$), suggesting improved mucosal integrity and secretion of protective mucus. These structural changes suggest enhanced nutrient absorption capacity and improved intestinal barrier function, reflecting a parallel increase in immune response and zootechnical parameters.

Furthermore, Slab51 supplementation improved growth performance and feed utilization indices, as reflected in higher weight gain and better FCR and PER values compared to the control group. The immune response also improved, with spleen macrophages from treated fish showing significantly higher respiratory burst activity ($p = 0.0167$), indicating a strengthened innate immune system.

In conclusion, this study highlights the profound impact of Slab51 on the intestinal architecture of Nile tilapia, which plays a central role in enhancing nutrient absorption, growth, and immunity. These findings underscore the potential of Slab51 as an effective probiotic for improving aquaculture productivity and fish health, with particular emphasis on its ability to remodel and strengthen the intestinal morphology of Nile tilapia.

1. Galli, G. (FIAA). Top 10 species groups in global, regional and national aquaculture 2021.
2. Ramos, M. A. *et al.* Dietary probiotic supplementation improves growth and the intestinal morphology of Nile tilapia. *Animal* **11**, 1259–1269 (2017).
3. Etyemez Büyükdeveci, M., Cengizler, İ., Balcázar, J. L. & Demirkale, İ. Effects of two host-associated probiotics *Bacillus mojavensis* B191 and *Bacillus subtilis* MRS11 on growth performance, intestinal morphology, expression of immune-related genes and disease resistance of Nile tilapia (*Oreochromis niloticus*) against *Streptococcus iniae*. *Dev. Comp. Immunol.* **138**, 104553 (2023).
4. Standen, B. . T. *et al.* Modulation of the intestinal microbiota and morphology of tilapia, *Oreochromis niloticus*, following the application of a multi-species probiotic. *Appl. Microbiol. Biotechnol.* **99**, 8403–8417 (2015).

Reference Intervals and innovative parameters using Sysmex XN-2000V in horses

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The evaluation of the hemogram is a key component in diagnosing diseases and monitoring animal health. This study aims to establish reference intervals (RIs) for healthy horses using a next-generation hematology analyzer designed for veterinary medicine (Sysmex XN-2000V). In addition to conventional hemogram parameters, this instrument provides innovative indices such as immature reticulocyte fraction (IRF) and can determine platelet count by three methods: impedance (PLT-I), optical (PLT-O), and fluorescence (PLT-F). Moreover, a specific fluorescent platelet channel can determine an important thrombopoietic marker named immature platelets fraction (IPF).

For this study, hemograms from 50 clinically healthy adult horses were analyzed. Mean, median and standard deviation were assessed, and RIs were calculated using the percentile method (2.5%; 97.5%). The following RIs were determined: HCT (26.94–39.24 %), HGB (9.89–14.10 g/dl), RBC ($5.5\text{--}8.3 \times 10^6/\mu\text{L}$), MCH (15.52–17.90 g/dl), MCHC (34.16–37.40 g/dl), MCV (43.13–50.04 fL), RDW (19.4–22.27 %), RET-HE (18.90–24.50 pg), RETICULOCYTES (0.05–0.22 %), RPI (0.00–0.10), WBC ($4.50\text{--}8.70 \times 10^3/\mu\text{L}$), corrected WBC ($4.50\text{--}8.70 \times 10^3/\mu\text{L}$), BAS (0.00–0.10 $\times 10^3/\mu\text{L}$), EOS (0.00–0.30 $\times 10^3/\mu\text{L}$), LYMPH (1.30–3.47 $\times 10^3/\mu\text{L}$), MON (0.20–0.40 $\times 10^3/\mu\text{L}$), NEU (2.40–5.41 $\times 10^3/\mu\text{L}$), MPV (7.12–8.70 fL), PCT (0.08–0.13 %), PLT-I ($61.20\text{--}152.80 \times 10^3/\mu\text{L}$), PLT-O ($96.90\text{--}170.00 \times 10^3/\mu\text{L}$), PLT-F ($96.75\text{--}160.60 \times 10^3/\mu\text{L}$), HFR (0.00–11.40 %), IRF (0.00–11.8 %), LFR (59.95–100.00 %), MFR (0.00–2.14%), IPF (0.30–4.06 %), PDW (6.51–15.21 fL), P-LCR (4.32–15.21 %), IPF# ($0.4\text{--}4.14 \times 10^3/\mu\text{L}$).

Establishing species-specific RIs based on the analytical instrument used is essential for accurately assessing the health status of both healthy and diseased horses, covering both standard and innovative parameters.

[1] O. Jornet-Rius, M. Mesalles-Naranjo and J. Pastor, “Performance of the Sysmex XN-V hematology analyzer in determining the immature platelet fraction in dogs: A preliminary study and reference values”, *Vet. Clin. Pathol.*, vol 52, pp. 433-442, 2023

[2] K.R. Friedrichs, K.E. Harr, K.P. Freeman, B. Szladovits, R.M. Walton, K.F. Barnhart and J. Blanco-Chavez, “ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics”, *Vet. Clin. Pathol.*, vol. 41, pp. 441-453, 2012

[3] A. Gavazza, C. Fani, C. Masci and G. Rossi, “Performance of platelet determination using the Sysmex XN 2000V analyzer and reference intervals in horses”, *Proc. 26th ESVCP Congress*, Budapest, pp. 140, August 2024

[4] M. Quagliardi, L. Galosi, G. Rossi, A. Roncarati and A. Gavazza, “Reference Intervals (RIs) in Veterinary Medicine”, *ACTA IMEKO*, vol 13, pp 1 – 5, 2024

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Morphometric And Histopathological Evaluation Of Chickens' Immune Organs Following *In-Ovo* Probiotic And Postbiotic Administration

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Histopathological examination of lymphoid organs offers valuable insights into animal health, serving as a reliable method to assess immune responses. In birds, this involves microscopic examination of primary lymphoid organs [spleen, Bursa Fabricii (BF)], or mucosa-associated lymphoid tissues (e.g. GALT). Studies on chickens often focus solely on macroscopic evaluation of immune organs, considering absolute or relative weight (organ/body weight ratio) of the spleen or BF, to evaluate the effects of different treatments [1,2]. However, many factors can influence organ weight. For instance, BF size can be altered by infectious agents (e.g. Infectious Bursal Disease Virus, Marek's Disease Virus) or mycotoxins feed contamination. Consequently, histopathological evaluation and measurement of structural parameters can provide more accurate information. This study aimed to evaluate the immunostimulatory potential of two distinct probiotic or probiotic + postbiotics mixtures, administered *in ovo* at embryonic day 17.5. Ross 308 eggs were injected into the amniotic fluid as follows: control group (C) received 100 µL of Marek's disease vaccine; group P received 100 µL of Marek's disease vaccine combined with 1×10^5 CFU/100 µL of probiotics (FSG68/22); and group T received 100 µL of Marek's disease vaccine combined with 1.7×10^4 CFU/100 µL of faecal dry extract enriched with 1×10^5 CFU/100 µL of live probiotic bacteria (FSG68/22). After hatching, the three groups (45 animals each) were divided into three equal replicates. Chickens were reared for 42 days and, at the end of the cycle, 5 birds/replicate were randomly selected at slaughtering and spleen and BF collected and weighted. Organs were processed for histopathological analysis. In BF, the cortical-to-medullary area ratio was calculated in 10 lymphoid follicles/sample, and a 0-3 was applied in order to assess the activation of the follicles from a normal state to severe hyperplasia. In the caecal tonsils, the area of 5 lymphoid follicles was calculated based on their height and width. Results showed significant differences among the groups. The relative spleen weight was reduced in P ($p=0.0486$). Concerning BF, a reduction was observed for relative weight in P and T ($p<0.0001$), cortical-to-medullary ratio in T ($p=0.0060$) and histopathological scores of both treated groups ($p=0.0025$). The caecal tonsil areas were increased in T group ($p<0.0012$). The results of these measurements proved that *in ovo* treatments, and particularly when the probiotic + postbiotic mixture is used, can have a positive effect on the activation of the immune system.

[1] Raji, A. A., Mohammed, B., Oladele, S. B., Saidu, L., Jibril, A. H., & Cazaban, C. (2017). Bursa body index as a visual indicator for the assessment of bursa of Fabricius. *Journal of veterinary medicine and animal health*, 9(2), 32-38.

[2] Song, Y., Yu, J., Wang, B., Wen, Q., Zhong, Y., Wu, M., & Zheng, X. (2023). Effect of fecal microbiota transplantation on early intestinal immune function and histomorphology of immune organs in chicks. *Letters in Applied Microbiology*, 76(12), ovad140.

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A new approach to evaluate veterinary histopathological tissue sample with image and ultrasound data

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In many proliferative lesions, arising from superficial and deep tissues, macroscopic and microscopic evaluations are essential in the diagnosis (e.g. hypertrophy, hyperplasia, dysplasia or neoplasia). The term virtual biopsy is gaining ground. This would transform the biopsy from a physical and invasive act to a virtual act, while still providing very precise diagnostic and prognostic information, always considering the traditional physical biopsy as the gold standard. A innovative rotary stage-based scanning system and a manual ultrasound platform were used to obtain, respectively, image and ultrasound data of tissue lesions, fixed in buffered formalin and used for classical histopathological examination. Fifty-nine samples (49 dogs, 9 cats, 1 rodent) of various fixed pathological tissues were analyzed as follows: 33 skin, 9 subcutaneous, 9 mammary, 3 oral mucosal, 2 intestinal, 1 renal, 1 bladder, and 1 splenic lesions. Image data were collected with the customized semi-automatic scanning platform, and ultrasound data from the same neoformations were collected using the custom-assembled ultrasound platform. The morphometric findings acquired by the device were then compared with manual measurements of the same masses, using a caliper. Finally, the histological section of the proliferate tissue, mirroring the one scanned by the *device*, was microscopically evaluated by expert pathologists, and a correct diagnosis was issued. The set of numerical data collected by the device was then compared with the morphometric data collected by the pathologist, to interpret the examined mass in terms of cellular damage and proliferative pattern. A 16 MHz high-frequency ultrasound probe was used to collect data, which allowed for greater resolution and more precise measurement of the target tissues. The quality of the collected data was assessed based on the clear discernibility of a reflection peak in the received ultrasound signal. A link between ultrasound and image data obtained from the camera can be established by identifying the scanner image whose position is closest to the ultrasound image. Finally, a data archive was created to process and visualize the obtained data set. Results show interesting indications that can be extrapolated from the data set, allowing to identify, based on the specific acoustic properties of the examined tissue or on the depth of the evaluated area, high quality signals that in the case of neoplasms allowed to distinguish the pathological areas from the healthy ones. Although preliminary, these data demonstrate that potential surrogates of macro-and microscopic assessments of tissue proliferations can be pursued through the new imaging metrics.

- [1] N. Brancati, A.M. Anniciello, P. Pati, D. Riccio, et al, "Bracs: A dataset for breast carcinoma subtyping in h&e histology images", *Database (Oxford)*, 2022, baac093.
- [2] O. Gemeinhardt, F.G. Poch, B Hiebl, U. Kunz-Zurbuchen, et al, "Comparison of bipolar radiofrequency ablation zones in an in vivo porcine model: Correlation of histology and gross pathological findings", *Clin. Hemorheol. Microcirc.*, vol 64, pp 491-499, 2016.
- [3] S.A. Goss, R.L. Johnston and F. Dunn, "Comprehensive compilation of empirical ultrasonic properties of mammalian tissues", *The J. Acoust. Soc. Am*, vol 64, pp. 423-457, 1978
- [4] J. Hau, "Animal models for human diseases: an overview", *Sourceb. models for biomedical research*, pp. 3-8, 2008.
- [5] A. Suvarna, R. Vempati, R. Chacko, G. Srinivasan, et al, "DeltaAI: Semi-autonomous tissue grossing measurements and recommendations using neural radiance fields for rapid, complete intraoperative histological assessment of tumor margins", *bioRxiv preprint* doi: <https://doi.org/10.1101/2023.08.07.552349>, 2023.

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Digital Immunohistochemical Quantification of Neu5Gc Expression in Canine Intestinal Biopsies and its Correlation with Colitis Severity

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Immunohistochemistry (IHC) is a powerful method for identifying the expression of specific antigens in formalin-fixed, paraffin-embedded tissues, extensively utilised in both diagnostics and research [1,2]. Despite the existence of numerous manual scoring systems documented in the literature, the decision process can be susceptible to visual bias: antibody amount, counterstaining density or brightness can influence this process. Digital processing systems have emerged as a solution to address these limitations, enabling quantitative analysis of diaminobenzidine (DAB) colour intensity. This approach not only reduces operator bias but also offers significant advantages, particularly in cases where markers exhibit diffuse staining in target tissue, resulting in distinct intensity variations corresponding to varying concentrations of the marker across a cell population [3]. In this study, we evaluated the intensity of N-glycolylneuraminic acid (Neu5Gc) expression using a digital scoring method, with the aim of correlating the marker expression to the grade of colitis. N-glycolylneuraminic acid (Neu5Gc) is a sialic acid synthesized from its N-acetyl precursor (Neu5Ac) by cytidine-5'-monophospho-N acetylneuraminic acid hydroxylase (CMAH). Absent in humans and ferrets, it is polymorphic in dogs. Loss of the CMAH gene generate a change in the structural profile of glycans of all tissues inducing the production of antibodies against Neu5Gc-glycans. Prolonged uptake of Neu5Gc by negative-CMAH dog through red meat and dairy products from +CMAH mammals leads to a progressive Neu5Gc-glycans incorporation in hosts' tissue (xenosialization), particularly if the gut microbiota is altered by de-sialilating bacteria, determining an inflammatory reaction, called "xenosialitis". For IHC analyses, colic biopsies belonging to European, Asian and American canine breeds (10 dogs/group) were obtained from the Archive of the Laboratory of Veterinary Pathology of the University of Camerino. An anti-Neu5GC chicken polyclonal antibody was used (Creative Diagnostic, DMABH-C003). The mean intensity of immunoreaction was measured in 10 microscopic fields at 40X magnification using the NIS Elements (Nikon) software. In this semiquantitative scoring system, 0 (white) represents the total absence of immunoreaction of the negative control, while 256 (black) the highest value of immunoreaction. The average values were calculated for each sample. A severity score (0–3) for intestinal colitis was assigned based on the histopathological evaluation of Haematoxylin-Eosin-stained biopsies, and these values were then compared to the IHC results. The expression of Neu5Gc was found to be entirely absent in the stomach and small intestine. The quantification of marker expression demonstrated a positive correlation between Neu5Gc expression and the degree of inflammation in the colon.

[1] Schacht V, Kern JS. Basics of immunohistochemistry. *J Invest Dermatol*. 2015;135: e30. Varghese F, Bukhari AB, Malhotra R, et al. IHC Profiler: an open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. *PLoS One*. 2014;9: e96801.

[2] Varghese F, Bukhari AB, Malhotra R, et al. IHC Profiler: an open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples. *PLoS One*. 2014;9: e96801.

[3] Varghese, Frency, et al. "IHC Profiler: an open source plugin for the quantitative evaluation and automated scoring of immunohistochemistry images of human tissue samples." *PLoS one* 9.5 (2014): e96801.

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Prevalence and antimicrobial resistance profiles of bacteria recovered from wounds of stray dogs and cats before and after washing with sterile physiological solution.

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Figure 1 – Sampling with sterile swab of traumatic wound infection before (T1) and after (T2) physiological solution washing.

Contaminated and infected wounds are highly prevalent in veterinary medicine [1,2]. Sterile physiological solution, a nontoxic isotonic solution, is the most commonly used cleansing agent in hospitals [3]. During May 2022 to December 2023, stray cats (n=16) and dogs (n=15) with traumatic or polytraumatic injuries were selected for this study, with sampling conducted under deep sedation prior to surgical wound debridement. Animals receiving antimicrobial treatments prior to sampling were excluded from the study.

The primary aim of this investigation was to compare the diversity of bacterial cultures obtained from swabs collected before and after washing with sterile physiological solution. Swabs were initially incubated in enrichment broth (BHI) for 24 hours at 37°C, followed by plating on various culture agar media for isolating Gram-positive and

Gram-negative bacteria. Species identification was performed via proteomic analysis (MALDI-TOF MS). The secondary objective focused on antimicrobial susceptibility testing, conducted using 18 antibiotics for Gram-positive and 16 antibiotics for Gram-negative bacteria.

Bacteria isolated from 61.3% (19/31) of the sampled animals remained consistent before and after washing. Among canine samples, *Staphylococcus pseudintermedius* was the most common bacterial strain (15 isolates), of which 80% (12/15) exhibited phenotypic resistance to oxacillin, penicillin, and cefoxitin, confirmed by the presence of the *mecA* gene detected by PCR. Feline wound samples revealed a broader variety of both Gram-positive and Gram-negative bacterial species, with a total of 75 isolates recovered. Notably, 40% of these isolates were identified as multidrug-resistant (MDR) strains.

These findings suggest that *i)* the viable microbial community in wounds remains largely unchanged following physiological solution washing, and *ii)* stray cats and dogs may serve as reservoirs of MDR bacteria, highlighting their potential role as indicators of environmental health.

[1] Bohling M.W., Henderson R.A. Differences in cutaneous wound healing between dogs and cats. *Vet Clin North Am Small Anim Pract.* 2006, 36(4):687-92.

[2] Kouamo J., Manie S.B., Kana A.G.D. Typology and management of wounds in dogs and cats in veterinary clinics in the city of Yaoundé, Cameroon. *Rev Vet Cli.* 2022, 57(4):149-165.

[3] Balsa I.M., Culp W.T. Wound Care. *Vet Clin North Am Small Anim Pract.* 2015, 45(5):1049-65.

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Methodological Framework to Study the Antibiotic Resistance Evolvability of Pathogenic Staphylococcal Species

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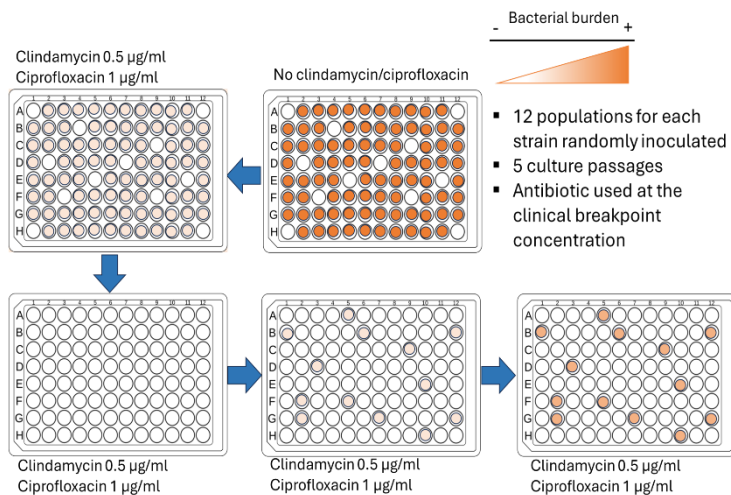


Figure 1 – Scheme of the experimental procedure to study the antibiotic resistance evolvability of staphylococci.

The increasing prevalence of antibiotic-resistant infections highlights the urgent need for novel antimicrobial strategies. While most research focuses on developing alternative therapeutics against resistant pathogens, an alternative approach involves addressing the evolutionary mechanisms driving resistance in antibiotic-susceptible bacteria. By preventing the emergence of resistance, this strategy could help preserve the efficacy of existing antibiotics. However, resistance evolvability remains poorly understood, and standardized methodologies for its investigation are lacking. In this study, we aimed to establish a methodological framework for assessing the resistance evolvability of major staphylococcal species. We evaluated the resistance evolvability of 61 fully susceptible *Staphylococcus pseudintermedius* isolates to clindamycin and 20 isolates to ciprofloxacin, all derived from canine clinical

samples. Our methodology was adapted from Papkou et al. [1], with fluctuation tests used to ensure that bacterial inocula were free from preexisting resistance mutations. The experimental setup, illustrated in Figure 1, was designed to mimic *in vivo* treatment conditions by exposing bacterial populations to clinical breakpoint concentrations of clindamycin (0.5 µg/ml) and ciprofloxacin (1 µg/ml) [2]. After four days of antibiotic exposure, evolved populations were identified based on optical density measurements (OD₅₉₅) relative to blank controls. Fluctuation test results supported the use of a bacterial inoculum of 3×10^5 cells/well (mutation rate 6.7×10^9 per cell division), aligning with previous findings [1]. Overall, *S. pseudintermedius* exhibited a low propensity for resistance evolution. Clindamycin resistance emerged in 17 out of 61 strains (25.4%), with varying degrees of evolvability: one strain exhibited 9/12 evolved populations, one 5/12, one 4/12, two 3/12, seven 2/12, and five 1/12. No resistance evolution was observed for ciprofloxacin. In contrast, prior studies reported significant resistance evolution in *S. aureus* at ciprofloxacin clinical breakpoint concentrations [1]. This suggests that *S. pseudintermedius* has a lower intrinsic capacity to evolve resistance to these antibiotics compared to *S. aureus*. Our experimental setup involved four days of serial antibiotic exposure, consistent with previous studies [1]. While this duration aligns with the minimum recommended treatment course for ciprofloxacin, it is shorter than the standard regimen for clindamycin. Ongoing investigations are focused on differential gene expression analysis between high- and low-evolvability *S. pseudintermedius* strains following clindamycin exposure. This research aims to identify key drivers of resistance evolution and support the development of anti-evolution strategies targeting the mechanisms underlying resistance acquisition.

- [1] A. Papkou, J. Hedge, N. Kapel, B. Young, and R. C. MacLean. “Efflux pump activity potentiates the evolution of antibiotic resistance across *S. aureus* isolates” *Nat. Commun.* Vol 11, pp. 3970, 2020.
- [2] Clinical and Laboratory Standards Institute “Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals”, CLSI, 2020.

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Challenges in Measuring the Antimicrobial Activity of Natural Compounds in *In Vitro* tests

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Over the past few years, the search for antimicrobials derived from natural compounds has intensified due to the growing bacterial resistance to conventional antibiotics and the lack of new, clinically effective antibiotics [1]. Natural plant-based substances are numerous and chemically diverse, encompassing flavonoids, polyphenols, alkaloids, coumarins, and essential oils [2]. Recently, a significant number of studies have focused on natural compounds as potential antibacterial agents. However, comparing results across studies is often challenging due to the use of various non-standardized *in vitro* techniques [3]. According to official guidelines for traditional antibiotics, antimicrobial susceptibility testing is standardized, ensuring consistency at every step—from antibiotic solubility conditions, polarity, inoculum preparation and size, growth medium, the inclusion of controls, to incubation conditions and the final determination of results for both agar diffusion and broth dilution methods [4].

In this study, we evaluated the antimicrobial activity of (i) honey and propolis; (ii) hazelnut extracts; and (iii) polyphenolic compounds from olive oil mill wastewater against relevant animal and food-borne pathogens. For all the compounds tested, antimicrobial activity was assessed using disk diffusion and broth dilution methods. The minimum inhibitory concentration (MIC), defined as the lowest concentration of extract that completely inhibited bacterial growth, was determined according to official guidelines [4]. Colonies were resuspended in saline solution, and their turbidity was measured spectrophotometrically (600 nm). The bacterial suspension used for the assay was 5×10^5 CFU/mL for MIC and 1×10^8 CFU/mL for disk diffusion. Plates were incubated under the specific bacterial growth conditions, and readings were taken after 24 hours. All experiments were performed in triplicate.

For all the natural compounds tested, Kirby-Bauer disk diffusion provided inconsistent results. Agar diffusion studies are not ideal for assessing the activity of plant extracts due to the significant impact of compound polarity on the results [5]. The MIC for honey was determined according to the guidelines, yielding reliable results. However, hazelnut extracts, propolis, and polyphenolic compounds from olive oil mill wastewater exhibited issues during *in vitro* testing. Hazelnut extracts were strongly colored, while propolis and olive oil mill wastewater polyphenols caused turbidity and precipitation, complicating the measurement of bacterial growth endpoints. For this reason, we also employed the agar dilution method combined with MIC evaluation.

In conclusion, no single method is ideal for testing natural compounds; rather, a combination of methods is more appropriate. Plant-based substances have the potential to serve as alternatives to synthetic compounds, but further efforts are needed to address the lack of standardization in antimicrobial activity measurement to achieve reliable and comparable results.

[1] M. Bubonja-Sonje, S. Knezevic, M. Abram, "Challenges to antimicrobial susceptibility testing of plant-derived polyphenolic compounds" *Arh Hig Rada Toksikol* vol.71, pp 300-311, 2020

[2] S. Li, S. Jiang, W. Jia, T. Guo, F. Wang, J. Li. "Natural antimicrobials from plants: recent advances and future prospects" *Food Chemistry* 432, 137231, pp 1-15, 2024.

[3] M. Balouiri, M. Sadiki, S. K. Ibensouda, "Methods for in vitro evaluating antimicrobial activity: a review," *Jour. Pharmaceutic Analysis*, vol. 6, pp. 71–79, 2016.

[4] CLSI. "Performance standards for antimicrobial susceptibility testing". CLSI guideline M100. *Clinical and Laboratory Standards Institute*, 2024

[5] J.N.Eloff "Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results" *BMC Complement Altern. Med*, vol. 19, pp. 1–8, 2019.

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Proposal of a risk-based approach for defining the calibration frequency of remote temperature sensors in use at the laboratories of Istituto Zooprofilattico Sperimentale delle Venezie (IZSve)

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Istituto Zooprofilattico Sperimentale delle Venezie (IZSve) is a public health institution which operates in the fields of food safety, animal health and animal welfare with the aim of supporting the food production sector and official sanitary controls. IZSve's laboratories are accredited under "ISO/IEC 17025:2017 - General requirements for the competence of testing and calibration laboratories".

Therefore, in order to satisfy the criteria established by the standard, IZSve shall ensure metrological traceability of its measurement results through periodical calibration of equipment, in order to guarantee accurate results within specified limits.

Relating to the temperature measurements, IZSve utilizes more than 1300 Pt100 wireless sensors that ensure a continuous monitoring for a range of laboratory equipment (e.g. refrigerators, incubators, freezers, room temperature), using a dedicated software.

Though the most recent guidelines [1] [2] contain some suggestions on the calibration frequency of remote temperature sensors, it is up to the laboratory to define the optimal intervals of verification, based on risk analysis.

To date, calibration is performed on annual basis. In order to balance the risk of obtaining unreliable results with the amount of resources to employ, IZSve is adopting an experimental method based on the calculation of the compatibility index (CI). The CI is calculated by comparing the equations that describe the performance of sensor probes over time.

So far, the obtained results indicate that the use of CI is a favourable approach for determining the calibration frequency of remote temperature sensors, ensuring that equipment meets user specifications. Indeed, whenever the calculated CI is below 1 ($CI < 1$), the calibration interval can be extended to a two year period, mitigating the risk of obtaining unreliable records.

[1] Calibration Guide EURAMET, cg-20, Version 3.0, (03/2011).

[2] Accreditation for Microbiological Laboratories, EURACHEM, Third edition, 2023.

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Development of an autogenous dip vaccine against Furunculosis (*Aeromonas salmonicida*) as tool for animal precision medicine and management: preliminary data.

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Furunculosis is an infectious disease sustained by *Aeromonas salmonicida*, that infects many freshwater and saltwater fish species, as Atlantic salmon (*Salmo salar*), char (*Salvelinus alpinus/Salvelinus fontinalis*), brown trout (*Salmo trutta fario*), marble trout (*Salmo trutta marmoratus*), grayling (*Thymallus thymallus*) and rainbow trout (*Oncorhynchus mykiss*).

Furunculosis manifest itself in different clinical forms: acute with high mortality, especially in fry, sub-acute with a typical septicaemic-haemorrhagic areas and chronic with typical skin lesions (furuncles). In addition to the abscess-like skin lesions/ulcers, often affecting the underlying musculature, *Aeromonas salmonicida* is capable of releasing strong exo-toxins.

The disease appears following stressful events (stocking densities, frequent sorting and balancing, handling, squeezing of broodstock, large temperature fluctuations) and it is difficult to manage, except by the antibiotics administration with the feed, obviously when the disease has been diagnosed.

Here we present the development of a new autogenous dipping vaccine intended for salmonid, in particular farmed addressed to rainbow trout. The precision immune treatment was prepared at Vaccine Unit of Pharmaceutical Dept. (IZSUM 'Togo Rosati', Perugia), while safety and efficacy tests were conducted at a trout farm located in Sefro, Macerata, Italy.

Approximately 1.5×10^6 rainbow fry, weighing less than 2 grams each, were treated with 2 inactivated vaccine varying antigen concentrations, by immersion in a reduced volume of water, for 60 minutes. The vaccination was performed under strict veterinary control and no adverse events were observed, confirming the product safety.

The efficacy was first assessed by the absence of new infection in the farm and secondly by repeated bacterial isolations and through MALDI-TOF identification. The vaccinated/control fry were moved 25 days after immunization into new tanks, exposed to the germ occurrence. For at least 60 days, the vaccinated animals were protected against infection, while in the control fry Furunculosis typical signs were found and *Aeromonas salmonicida* was isolated and identified. After 60 days, Furunculosis was also registered in some vaccinated fry. These outcomes suggested for the future the variation of two key-factors: the immunizing dose and the immunization protocol.

Further trials are necessary in order to validate a precision immunizing product, characterized by high coverage, long-lasting and protective properties.

In conclusion, it is necessary to develop further precision vaccines, in order to increase and upgrade the immune-proficiency. Autogenous custom-made and precision vaccination, may represent in perspective a valid and sustainable tool to manage infection in farmed salmonids.

RFID-Enabled Smart Livestock Management Systems

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(a)



(b)

Fig. 1. Photograph of a successful communication from the ASR PC software to the reader via router and internet, (b) reading is recorded when a bolus is placed inside the zone created by the two in phase antenna (inset) rumen bolus.

Agriculture 4.0, as a part of Industry 4.0, completely emerged into the smart collection of data and its use for bene-fit of farm animals [1]-[2]. Nowadays, RFID (Radio Frequency Identification) technology has added a crucial role in it, being used for tasks such as precise monitoring of livestock including tracking of animal's feeding and resting patterns, monitoring them during milk extraction, detecting diseases early and so on [3] - [5]. Low-Frequency RFID is particularly useful for cattle monitoring because it can penetrate materials like metal and biological tissues, with an additional benefit of slower tag read/ write rates for precise observation. However, its short read range often requires bigger external antennas and associated mechanical issues. Ultra-High Frequency (UHF) RFID has longer read range and the ability to process multiple tags simultaneously which make it ideal for large-scale precision livestock farming.

This work discusses the development of an RFID-based gate system, operating at 124.2/134.2 kHz, for milking parlours to automatically track cattle boluses, identify and count animals, and maintain a real-time database to improve farm management. The novelty lies in setting a wireless monitoring channel along with providing a fair enough area of detection by adjusting the RF antennas and transmitter. The reader uses Lector LF ASR650 motherboard, a wireless router, Wi-Fi/Bluetooth monopole antennas and two loop antenna Panel each of 50x60 cm [6]. The router and the antennas help to establish a wireless connection of tracking and database collecting software (ASR-PC-Demo AGRIDENT V 1.84.01) [7] from the reader to PC as depicted in Fig. 1a. The system is being tested under ideal laboratory conditions, placing the rumen bolus inside human hands and passing through the gate (Fig. 1b). The results showed high accuracy in identification where (i) the two antennas are far apart up to 90 cm, and (ii) the operator monitoring distance is up to 1-2 meter far from the RFID system through wireless channel.

Moreover, Ultra-High Frequency (UHF) RFID for posture sensing will be discussed. Due to its longer read range and ability to support multiple tag reads simultaneously, UHF RFID can be integrated with sensor-based systems to monitor animal movement, posture, and activity levels in real time. This capability enhances precision livestock farming by enabling early detection of lameness, fatigue, or abnormal behaviours.

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- [1] Morrone S, Dimauro C, Gambella F, Cappai MG. Industry 4.0 and Precision Livestock Farming (PLF): An up to Date Overview across Animal Productions. *Sensors*. 2022; 22(12):4319.
- [2] L.M. Kamarudin, A. Zakaria, M.N. Rahman, et al., Monitoring feeding and resting pattern of goats in dairy farm using long-range RFID-based system, in *Proceedings of the 7th International Conference on Communications and Broadband Networking (2019)*, pp 41–45.
- [3] Aungier, S. P. M., Roche, J. F., Sheehy, M., & Crowe, M. A. (2012). Effects of management and health on the use of activity monitoring for estrus detection in dairy cows. *Journal of Dairy Science*, 95(5), 2452-2466.
- [4] Nieddu, G. (2017). Electronic identification of sheep in Sardinia: a retrospective analysis of the past fifteen years.
- [5] John, A. J., Clark, C. E. F., Freeman, M. J., Kerrisk, K. L., Garcia, S. C., & Halachmi, I. (2016). Milking robot utilization, a successful precision livestock farming evolution. *Animal*, 10(9), 1484-1492.
- [6] <https://www.agrident.com/products/stationary-readers/asr650/>
- [7] <https://www.agrident.com/downloads/dealer-abr200-software/>

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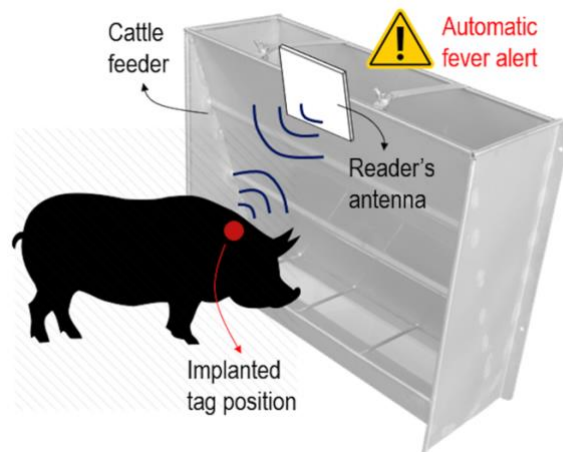
Battery-less Implantable Wireless Sensor for Proactive Fever Monitoring in Livestock

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Timely detection of fever in livestock remains a critical challenge in animal health management [1], particularly due to the impracticality of traditional rectal temperature measurements in large populations. This paper presents a battery-less implantable wireless sensor designed for the automatic monitoring of core temperature in pigs, with potential applications in broader livestock management. The proposed sensor employs a uniquely shaped loop antenna integrated onto a meshed textile scaffold, which provides an anti-migration effect and ensures natural integration with the animal's subcutaneous tissue. Operating under the UHF-RFID protocol, the device can be interrogated from distances up to 1.7 meters, thereby enabling seamless, automatic temperature monitoring as animals approach feeding stations. The innovation lies in merging RFID technology with temperature sensing [2] within a thin, flexible implantable device, optimized to maintain reliable communication performance despite the challenges imposed by tissue propagation effects. Extensive simulations have guided the antenna design and its integration with a self-tuning RFID IC, while experimental evaluations using a pork neck phantom have validated the system's efficacy. The results indicate that the sensor not only meets the performance requirements for effective fever detection but also offers a promising solution to reduce the reliance on broad-spectrum antibiotic treatments by facilitating targeted interventions based on real-time health data.



[1] L. Lohse, A. Uttenthal, C. Enøe, and J. Nielsen, *Acta Veter. Scand.*, vol. 52, p. 29, May 2010.2.

[2] M. C. Caccami and G. Marrocco, *IEEE Trans. Antennas Propag.*, vol. 66, no. 6, pp. 2779–2787, Jun. 2018

UHF-RFID Localization for sheep monitoring in milking parlour

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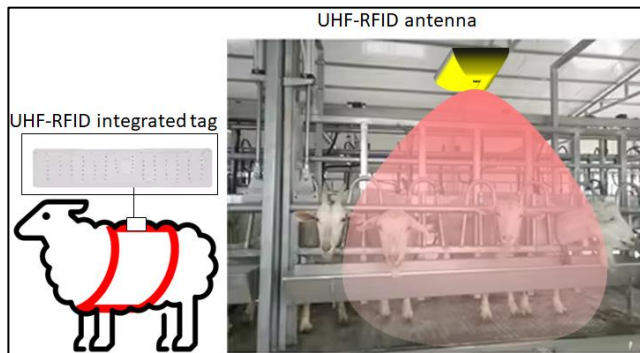


Figure 1 – Example of UHF-RFID Localization System within a milking parlour.

Animal identification is one of the pioneering applications of the Radio Frequency Identification Technology (RFID) [1]. Among the first solutions were the boluses that reside in the pre-stomachs of ruminants. The bolus is equipped with a passive Low Frequency (LF) tag which consists of a loop connected to a microchip. The operating frequency is 125.4 kHz, or 134 kHz more rarely, thus allowing the detection even in presence of organic materials. Indeed, the communication occurs through magnetic coupling and tag sends its unique identifier through a load modulation principle. The reading range is limited to few centimeters, so typically, a portable reader or a gate are employed. As alternative, ear tags can be adopted.

They have been designed to operate in the LF bandwidth too. In this case the form is circular due to the loop antenna, so the tag adheres completely to the earlobe. Also rectangular ear tags have been developed in the Ultra High Frequency (UHF) band, i.e., 860-930 MHz. This shape causes the dipole-like tag to hang from the ear so it can radiate in free space by guaranteeing good functionality. Indeed, at this frequency, the performance is affected by the presence of liquids and organic materials that reduce the reading range, to the point of compromising the detection. Instead a tag that protrudes from the earlobe allows a reading range of few meters. The latter solution enables more flexibility in the management of the sheeps for example during the milking operations. Indeed, the enhanced reading range allows to simultaneously detect all sheeps within the stable. Moreover, it is possible to implement a UHF-RFID tracking system [2] to also monitor the sheep position and associate it to the milking station. The latter information is crucial to associate the milk quantity produced by each sheep.

This abstract proposes the adoption of UHF-RFID localization for the management of the sheep during milking. By considering the parlour structure, it is possible to use an antenna array capable of electronic beam switching installed at the ceiling. As alternative, the employment of synthetic array approach [3] with a UHF-RFID antenna moving above the sheep is considered, if it is possible to use at least a linear antenna motion system. The effect of the animal body on the tag detection has been investigated, by using a passive tag installed on a smart harness, similarly to that employed for laundry applications.

[1] R. E. Floyd, "RFID in Animal-Tracking Applications," in *IEEE Potentials*, vol. 34, no. 5, pp. 32-33, Sept.-Oct. 2015.

[2] E. Pereira et al., "RFID Technology for Animal Tracking: A Survey," in *IEEE Journal of Radio Frequency Identification*, vol. 7, pp. 609-620, 2023.

[3] A. Buffi, A. Motroni, P. Nepa, B. Tellini and R. Cioni, "A SAR-Based Measurement Method for Passive-Tag Positioning With a Flying UHF-RFID Reader," in *IEEE Transactions on Instrumentation and Measurement*, vol. 68, no. 3, pp. 845-853, March 2019.

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