

# Book of Abstracts

of the 4th EAAP Regional Meeting 2026



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**Sassari – Italy**  
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**20-22 May, 2026**



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# The European Federation of Animal Science (EAAP)

The main aims of the EAAP are to promote, by means of active co-operation between its members and other relevant international and national organisations, the advancement of scientific research, sustainable development and production systems; experimentation, application and extension; to improve the technical and economic conditions of the livestock sector; to promote the welfare of farm animals and the conservation of the rural environment; to control and optimise the use of natural resources in general and animal genetic resources in particular; to encourage the involvement of young scientists and technicians. More information on the organisation and its activities can be found at [www.eaap.org](http://www.eaap.org).

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The European Federation of Animal Science (EAAP) has close established links with its sister organizations of American Society of Animal Science (ASAS), American Dairy Science Association (ADSAS), Canadian Society of Animal Science (CSAS) and Asociación Latinoamericana de Producción Animal (ALPA) and is also member of the World Association for Animal Production (WAAP).



# Welcome to the 4<sup>th</sup> EAAP Regional Meeting in Sassari

On behalf of the Organizing Committee of the University of Sassari, it is our honor and pleasure to welcome you to the 4th EAAP Regional Meeting, scheduled from May 20 to 22, 2026, in the city of Sassari, Italy.

This event represents a valuable opportunity to bring together scientists, researchers, and professionals from across Europe and beyond to address the key challenges of animal production in a rapidly changing world.

The scientific program will focus on major areas of relevance for the livestock sector, including animal genetics and the management of genetic resources, sustainable animal production systems, and innovations in precision livestock farming and digital technologies. Particular attention will also be given to animal nutrition and its role in improving productivity, efficiency, and environmental sustainability.

Sassari, with its rich historical and cultural heritage, provides an inspiring setting for this meeting. Participants will have the opportunity not only to engage in a stimulating scientific program but also to explore the city's traditions, nearby archaeological sites such as Nuraghe Santu Antine and *Complesso nuragico di Palmavera*, and the unique landscapes of northern Sardinia.

We are confident that the 4th EAAP Regional Meeting will be a dynamic and engaging event, fostering collaboration, knowledge exchange, and innovation. We look forward to welcoming you to Sassari for a productive and inspiring experience.

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# Organizers of the 4<sup>th</sup> EAAP Regional Meeting

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## About the University of Sassari



The University of Sassari, Italy, is a prestigious institution dedicated to research and education in agricultural sciences and related fields. Founded in 1562, it has a long and distinguished history of contributing to advancements in agriculture, environmental protection, and rural development. The university's mission is to provide high-quality education while fostering research that addresses contemporary challenges in agriculture and food production. As a key player in Italy's agricultural research landscape, the university focuses on a variety of disciplines, including animal science, plant breeding, horticulture, and agricultural economics. It comprises numerous experienced scientists who engage in innovative projects, collaborating with national and international research networks to enhance knowledge transfer and application in the agricultural sector. The University of Sassari is committed to sustainability and environmental stewardship, working to develop practices that support both agricultural productivity and the preservation of natural resources. The institution offers comprehensive educational programs that equip students with the necessary skills to address global agricultural challenges. In addition to its educational and research activities, the university maintains modern laboratories and research facilities, providing a conducive environment for cutting-edge studies.

For more information about the University of Sassari, you can visit their official website <https://www.uniss.it/>.

# EAAP Study commissions



## Commission on Animal Genetics

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Marcin Pszczola	Vice-President	Poznan University of Life Sciences, Poland	marcin.pszczola@gmail.com
Morten Kargo	Vice-President	Aarhus University, Denmark	morten.kargo@qgg.au.dk
Ewa Strucken	Vice-President	Humboldt-Universität zu Berlin, Albrecht Daniel Thaer Institut, Germany	-
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Francesco Tiezzi Mazzoni Della Stella Maestri	Secretary	University of Florence, Italy	francesco.tiezzi2@unifi.it
Christa Egger-Danner	Industry rep.	Zuchtdata, Austria	egger-danner@zuchtdata.a
Derek Bickhart	Industry rep.	Hendrix Genetics, Netherlands	-
Giuseppina Schiavo	Young Club	University of Bologna, Italy	giuseppina.schiavo2@unibo.it
Pauline Martin	Young Club	INRAè, France	-
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Maria José Ranilla García	President	Universidad de León, Spain	mjrang@unileon.es
Luciano Pinotti	Vice-President	University of Milan, Italy	luciano.pinotti@unimi.it
Susanne Kreuzer- Redmer	Vice-President	Vetmed Vienna, Austria	susanne.kreuzer-redmer@vetmeduni. ac.at
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Sokratis Stergiadis	Secretary	University of Reading, UK	s.stergiadis@reading.ac.uk
Javier Alvarez Rodriguez	Secretary	University of Zaragoza, Spain	javier.alvarezr@unizar.es
Kar Soumya	Secretary	Wageningen University & Research, Netherlands	soumya.kar@wur.nl
Yron Joseph Manaig	Industry rep	ANIMINE, France	yronjosephmanaig@gmail.com
Maria Soledad Gomez Conde	Industry Rep	ADM Animal Nutrition, Spain	mariosoledad.gomezconde@adm.com
Lynda Perkins	Young Club	Queen's University Belfast, UK	-
Giulia Foggi	Young Club	University of Pisa, Italy	giulia.foggi@agr.unipi.it



## Commission on Health and Welfare

Laura Boyle	President	Teagasc, Ireland	<a href="mailto:laura.boyle@teagasc.ie">laura.boyle@teagasc.ie</a>
Mariana Dantas de Brito Almeida	Vice President	University of Tras-os-Montes and Alto Douro, Portugal	<a href="mailto:mdantas@utad.pt">mdantas@utad.pt</a>
Mirjam Holinger	Vice President	Research Institute of Organic Agriculture FIBL, Switzerland	<a href="mailto:mirjam.holinger@fibl.org">mirjam.holinger@fibl.org</a>
Isabel Blanco Penedo	Secretary	University of Lleida, Spain	<a href="mailto:isabel.blancopenedo@udl.cat">isabel.blancopenedo@udl.cat</a>
Angela Trocino	Secretary	University of Padova, Italy	<a href="mailto:angela.trocino@unipd.it">angela.trocino@unipd.it</a>
Liat Morgan	Secretary	University of Tel Aviv Israel	<a href="mailto:liatmorgan@gmail.com">liatmorgan@gmail.com</a>
Barbara Folis	Secretary	University of Veterinary Medicine Vienna, Austria	-
Guilherme A. Franchi	Young Club	Aarhus University, Denmark	<a href="mailto:gaf@anivet.au.dk">gaf@anivet.au.dk</a>
Flávio Daniel Gomes da Silva	Young Club	Tras-o-Montes e Alto Duoro University, Portugal	<a href="mailto:fsilva@uevora.pt">fsilva@uevora.pt</a>
Vincenzo Lopreiato	Young Club	University of Messina Italy	<a href="mailto:vincenzo.lopreiato@unime.it">vincenzo.lopreiato@unime.it</a>
Naomi Duijvesteijn	Industry rep	Hendrix Genetics, Netherlands	<a href="mailto:Naomi.Duijvesteijn@hendrix-genetics.com">Naomi.Duijvesteijn@hendrix-genetics.com</a>



## Commission on Animal Physiology

David Kenny	President	Teagasc, Ireland	<a href="mailto:david.kenny@teagasc.ie">david.kenny@teagasc.ie</a>
Kate Keogh	Vice-President	Teagasc, Ireland	<a href="mailto:kate.keogh@teagasc.ie">kate.keogh@teagasc.ie</a>
Marco Tretola	Secretary	Agroscope, Switzerland	<a href="mailto:marco.tretola@agroscope.admin.ch">marco.tretola@agroscope.admin.ch</a>
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Franziska Koch	Secretary	Research Institute for Farm Animal Biology, Germany	<a href="mailto:koch@fbn-dummerstorf.de">koch@fbn-dummerstorf.de</a>
Olaia Urrutia	Young Club	University of Navarra, Spain	<a href="mailto:olaia.urrutia@unavarra.es">olaia.urrutia@unavarra.es</a>



## Commission on Livestock Farming Systems

Enrico Sturaro	President	University of Padova, Italy	enrico.sturaro@unipd.it
Ioanna Pouloupoulou	Vice President	Agricultural University of Athens, Greece	ioanna.pouloupoulou@aau.gr
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Tiago T. da Silva Siqueira	Secretary	CIRAD, France	tiago.teixeira_da_silva_siqueira@cirad.fr
Saheed Salami	Industry Rep.	Adisseo, France	sayodeji14@yahoo.com
Sarah Morgan	Young Club	Harper Adams University, UK	-



## Commission on Cattle

Massimo De Marchi	President	Padova University, Italy	massimo.demarchi@unipd.it
Carmen L. Manuelian	Vice-President	Autonomous University of Barcelona, Spain	carmen.manuelian@uab.cat
Adam Cieslak	Vice-President	Poznan University of Life Sciences, Poland	adam.cieslak@up.poznan.pl
Alberto Cesarani	Secretary	Sassari University, Italy	acesarani@uniss.it
Eduard Coudert	Industry Rep.	CMI Roullier, Francia	-
Saskia Meier	Industry Rep.	SYNETICS Germany Gmb Germany	saskia.meier@synetics.world
Alper Tuna Kavlak	Young Club	Natural Resources Institute Finland (Luke), Finland	alper.kavlak@luke.fi
Giulio Giagnoni	Young Club	Aarhus University, Denmark	gigi@anivet.au.dk



## Commission on Sheep and Goat Production

Georgia Hadjipavlou	President	Agricultural Research Institute, Cyprus	ghadjipavlou@ari.moa.gov.cy
Lorenzo E. Hernández Castellano	Vice-President	Universidad de Las Palmas de Gran Canaria, Spain	lorenzo.hernandez@ulpgc.es
Antonello Cannas	Secretary	University of Sassari, Italy	cannas@uniss.it
Siham A. Rahmatalla	Secretary	Albrecht Daniel Thaer Institute for Agricultural and Horticultural Sciences, Germany	rahmatas@hu-berlin.de
Francis Campion	Secretary	Teagasc, Ireland	francis.campion@teagasc.ie
Ebru Emsen	Industry Rep	ERGEN R&D company, Turkey	ebru.emsen@er-gen.com
Christos Dadousis	Young Club	University of Surrey, UK	c.dadousis@surrey.ac.uk



## Commission on Pig Production

Paolo Trevisi	President	Bologna University, Italy	paolo.trevisi@unibo.it
Tristan Chalvondemersay	Vice President	ADM, France	tristan.chalvondemersay@roullier.com
Marion Girard	Secretary	Agroscope, Switzerland	marion.girard@agroscope.admin.ch
Peadar Lawlor	Secretary	Teagasc, Ireland	peadar.lawlor@teagasc.ie
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Lisanne Verschuren	Young Club	Topigs Norsvin, The Netherlands	lisanne.verschuren@topignorsvin.com



## Commission on Horse Production

Celine Vial	President	INRAe, France	celine.vial@inrae.fr
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Roberto Mantovani	Vice-President	University of Padua DAFNAE, Italy	roberto.mantovani@unipd.it
Pasquale De Palo	Vice-President	University of Bari, Italy	pasquale.depalo@uniba.it
Jackie Tapprest	Secretary	Animal health laboratory (ANSES), France	jackie.tapprest@anses.fr
Emanuela Valle	Secretary	Torino University, Italy	emanuela.valle@unito.it
Claire Neveux	Industry Rep	Ethonova, France	ethonovaep2i@gmail.com
Samy Julliard	Industry Rep	Lab to field, France	amy.julliard@lab-to-field.com
Kirsty Tan	Young Club	Christian-Albrechts-Universität zu Kiel, Germany	kirsty.tan89@gmail.com
Samanta Mecocci	Young Club	University of Tuscia, Italy	-



## Commission on Precision Livestock Farming

Jarissa Maselyne	President	ILVO, Belgium	jarissa.maselyne@ilvo.vlaanderen.be
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Maria-Anastasia Karatzia	Secretary	Research Institute of Animal Science, Greece	karatzia@elgo.gr
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Daniel Foy	Industry Rep	AgriGates, USA	d.foy@agrigrates.io
Michael Odintsov Vaintrub	Industry Rep	Regrowth s.r.l.s Italy	m.odintsov.vaintrub@gmail.com
Adrien Lebreton	Young Club	Institut de l'élevage, France	adrien.lebreton@idele.fr
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## Commission on Insects

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Ridmantas Zelionka	Industry Rep	ENTOGAMA, Lithuania	<a href="mailto:ridmantas@entogama.com">ridmantas@entogama.com</a>
Anton Gligorescu	Industry Rep	EntoPrime, Denmark	<a href="mailto:angl@bio.au.dk">angl@bio.au.dk</a>
Marisa Santos	Industry Rep	ThunderFoods, Portugal	<a href="mailto:marisa.santos@thunderfoods.pt">marisa.santos@thunderfoods.pt</a>
Marco Tulio Tejada	Industry Rep	Nasekomo, Bulgaria	-
Angelo Katsanis	Industry Rep	Farninsect, Germany	-
Christophe Trespeuch	Industry Rep	Mutatec France	<a href="mailto:c.trespeuch@mutatec.com">c.trespeuch@mutatec.com</a>
Sara Bellezza Oddon	Young Club	University of Turin, Italy	<a href="mailto:sara.bellezzaoddon@unito.it">sara.bellezzaoddon@unito.it</a>
Roel Meyermans	Young Club	KU Leuven, Belgium	-
Ilaria Biasato	Young Club	University of Turin, Italy	<a href="mailto:ilaria.biasato@unito.it">ilaria.biasato@unito.it</a>
Somaya Naser El Deen	Young Club	Wageningen Livestock Research, The Netherlands	<a href="mailto:somaya.nasereldeen@wur.nl">somaya.nasereldeen@wur.nl</a>



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# Scientific programme

## Session 1. Animal Genetic Resources: Local and Transboundary Breeds in the Mediterranean: Diversity Aspects for Sustainable Livestock Farming

Date: Wednesday 20 May 2026; 14:30 - 18:15

Chair: Bojkovski / Hadjipavlou / Ligda

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Chair: Foggi / Cannas

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### **Integrating phenotypic and genomic data to describe a Sicilian local cattle ecotype adapted to Mediterranean environments**

V. Floridia<sup>1</sup>, A. Bionda<sup>2</sup>, C. Cavallo<sup>1</sup>, V. Lopreiato<sup>1</sup>, P. Crepaldi<sup>2</sup>, L. Liotta<sup>1</sup>, M. Barbato<sup>1</sup>, G. P. Patti<sup>1</sup>

<sup>1</sup> University of Messina, Viale Giovanni Palatucci, 98168 Messina, Italy, <sup>2</sup> University of Milano, Via Celoria 2, 20133 Milano, Italy

Sicily hosts three local cattle populations: the officially recognized Modicana and Cinisara breeds, and the Siciliana ecotype, reared in the mountainous Nebrodi area. Socioeconomic changes, the lack of official recognition, and unrestricted crossbreeding with cosmopolitan breeds have accelerated genetic erosion, threatening the conservation of the Siciliana population. Here we combined phenotypic and genomic analyses to characterize the Siciliana cattle and gather information to support conservation strategies. Phenotypic data, including qualitative and morphometric traits, were collected from 32 individuals representative of the typical mountain ecotype. Genomic analyses were conducted using medium-density SNP data from 90 Siciliana individuals compared with 35 Italian and French breeds. Results showed a body conformation coherent with the adaptation to mountainous environments and extensive farming, characterised by a mesomorphic and dolichomorphic conformation, homogeneous qualitative traits, and a variable body types. Genomic analyses revealed moderate genetic diversity ( $H_o = 0.33$ ), low inbreeding (0.04), and a distinct profile of genomic ancestry, whilst still grouping with the other Sicilian breeds. Introgression varied among farms and reflected different breeding practices, including crosses with Limousine, to increase meat yield, or Modicana, likely to benefit from its recognized breed status. Overall, our results suggest the uniqueness of the Siciliana ecotype but highlight significant genetic erosion due to extensive crossbreeding, emphasizing the need for targeted conservation actions.

## Session 1

## Theatre 2

### **Evaluating landscape omics results in local sheep breeds from Mediterranean North African area**

M. Milanese<sup>1,2</sup>, E. Somenzi<sup>1</sup>, E. Capra<sup>3</sup>, P. Cozzi<sup>3</sup>, B. Lazzari<sup>1,3</sup>, B. Benjelloun<sup>4</sup>, A. Carta<sup>5</sup>, S. S. Gaouar<sup>6</sup>, A. Jannoune<sup>7</sup>, H. Khemiri<sup>8</sup>, N. M'Hamdi<sup>9</sup>, N. Tabet Aoul<sup>10</sup>, G. Zitouni<sup>11</sup>, M. Tixier-Boichard<sup>12</sup>, A. Stella<sup>3</sup>, P. Ajmone Marsan<sup>1</sup>

<sup>1</sup> UCSC, E Parmense 84, 29122 Piacenza, Italy, <sup>2</sup> CMCC, M Biagi 5, 73100 Lecce, Italy, <sup>3</sup> IBBA-CNR, A Corti 12, 20133 Milano, Italy, <sup>4</sup> INRA, Ennasr, 415 Rabat, Morocco, <sup>5</sup> AGRIS, Bonassai, 07100 Sassari, Italy, <sup>6</sup> PPABIONUT, Abi Ayad Abdelkrim, 13000 Tlemcen, Algeria, <sup>7</sup> ANOC, Hassan II, 10090 Rabat, Morocco, <sup>8</sup> ODESYANO, A. de l'Environnement, 9000 Béja, Tunisia, <sup>9</sup> INAT, Charles Nicolle, 1082 Tunis, Tunisia, <sup>10</sup> USTO, Bir El Djir, 31000 Oran, Algeria, <sup>11</sup> ITELV, Birtouta, 16000 Alger, Algeria, <sup>12</sup> INRAE, GABI, 75352 Jouy-en Josas, France

Following domestication in the Fertile Crescent, sheep spread globally and adapted to diverse environments through both artificial and long-term natural selection. Understanding how environmental factors shape genomic and epigenomic variation is essential for improving adaptation to climate change. The SCALA-MEDI project investigated DNA sequence variation and CpG methylation in local sheep breeds from Algeria, Morocco, and Tunisia reared under contrasting agroclimatic conditions, using landscape genomics and epigenomics. Two genomic datasets were analyzed: 753 individuals genotyped with the IMAGE0001V2 SNP array and 132 individuals sequenced at low coverage. Genome–environment association analyses were conducted using latent factor mixed models (LFMM) and partial redundancy analysis (pRDA) based on 19 bioclimatic variables and elevation from WorldClim. In parallel, DNA methylation was assessed in 147 animals from different Köppen–Geiger biomes using RRBS, and differentially methylated cytosines (DMCs) were identified with SeqMonk. SNP array analyses identified a signal associated with BIO15 (precipitation seasonality variability) in the HMGA2 gene, while WGS detected 80 additional SNPs associated with 6 bioclimatic variables and elevation. Epigenomic analyses revealed approximately 2,000 DMCs shared across biome comparisons. Notably, 23 genomic and epigenomic signals associated with 4 bioclimatic variables and elevation co-localized, suggesting coordinated genetic and epigenetic mechanisms underlying environmental adaptation. Functional annotation analyses are ongoing to identify the involved pathways.

**The Rhodope Shorthorn cattle - an example of revival of an ancient breed**

S. Nikolov<sup>1</sup>, Z. Ducheve<sup>2</sup>, M. Ignatova<sup>2</sup>, V. Nikolov<sup>1</sup>

<sup>1</sup> Agricultural University – Plovdiv, Department of Animal Sciences, Mendeleev blvd 12, 4000 Plovdiv, Bulgaria, <sup>2</sup> Institute of Animal Science, Agricultural Academy, Pochivka stn, 2232 Kostinbrod, Bulgaria

The Rhodope Shorthorn cattle is one of the two autochthonous breeds in Bulgaria preserved from ancient times to the present day. It belongs to the group of brachycerous cattle breeds (*Bos taurus brachyceros*) on the Balkans, which are considered among the oldest cattle populations in Europe. During the last century, the introduction of highly productive commercial breeds led to a severe decline in the population size of the Rhodope Shorthorn, with only a small number of animals maintained at the National Centre for Animal Husbandry and Agriculture in Smolyan. The revival of the breed began in 2001 through field expeditions aimed at identifying remaining animals in isolated villages near the Bulgarian–Greek border. In the subsequent years, the population size has grown rapidly and two breeding associations were established. At present, the majority of the population is concentrated in three regions of the Central and East Rhodope Mountains. Several farms are located north of Stara Planina Mountain, a natural epizootic barrier separating southern and northern Bulgaria. These populations represent an important genetic reserve which, combined with the substantial amounts of *ex situ* in vitro conserved reproductive material, could be used to restore the breed in case of disaster. The breed is an integral part of the Rhodope landscape and in recent years innovative approaches have been implemented to increase public awareness within the framework of two of the increasingly popular trail running competitions, organized in this region.

**Transboundary Cooperation in the Conservation of the Bosnian Mountain Horse: The Slovenian Case Study**

D. Bojkovski<sup>1</sup>, T. Flisar<sup>1</sup>, M. Mesarič<sup>2</sup>

<sup>1</sup> Biotechnical Faculty, University of Ljubljana, Department for animal science, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia, <sup>2</sup> Veterinary Faculty, University of Ljubljana, Gerbičeva 60, SI-1000 Ljubljana, Slovenia

Transboundary local breeds, shared between neighbouring countries, are an important component of animal genetic resources, combining broad ecological adaptation with cultural and socio-economic significance. In the Alpine and Dinaric regions of Slovenia, local breeds such as the Bovec sheep, Istrian Pramenka sheep, Posavje horse, Istrian cattle, and Bosnian mountain horse occur across national borders and are managed under a wide range of environmental and production conditions, making them valuable models for studying genotype–environment interactions and adaptive diversity. This paper presents a case study on the characterisation and conservation of a critically endangered transboundary population of the Bosnian mountain horse, with particular emphasis on genetic diversity and functional traits related to robustness and climate resilience, developed through long-term adaptation to mountainous environments and low-input pack and draught systems. Special attention is given to the combined use of phenotypic and genomic information for performance evaluation within a jointly coordinated international breeding and recording system. The contribution of local breeds to ecosystem services, extensive and circular production systems, and the maintenance of rural livelihoods in marginal areas is also addressed. In addition, the importance of harmonised recording systems, joint breeding programmes, and coordinated policy frameworks is emphasised as a prerequisite for the long-term conservation and sustainable use of these genetic resources.

### **Developing guidelines for transboundary breeding programmes to preserve endangered breeds in the European Union**

R. Bonifazi<sup>1</sup>, J. Ten Napel<sup>1</sup>, M. Schoon<sup>1</sup>, M. Spoelstra<sup>1</sup>, L. Balzar<sup>2</sup>, H. Göderz<sup>2</sup>, S. J. Hiemstra<sup>1</sup>

<sup>1</sup> Wageningen University & Research, Droevendaalsesteeg 2, 6708PB Wageningen, Netherlands, <sup>2</sup> BLE, Deichmanns Aue 29, 53179 Bonn, Germany

Genetic diversity in livestock is increasingly at risk. A recent study revealed that ~42% of national breed populations in Europe are transboundary, i.e. distributed in at least two countries. Out of these, ~23% are considered at risk in all countries. The practical implementation of (transboundary) breeding programmes for endangered breeds remains complex due to differences in management structures and (local) regulations. To address this issue, the European Union Reference Centre for Endangered Animal Breeds (EURC-EAB) is developing comprehensive guidelines to assist breed societies and national competent authorities in strengthening cross-border collaboration to improve the genetic diversity of transboundary breeds. To structure the decision-making process for establishing transboundary cooperation, we propose a novel "two-layer framework". The first layer assesses the biological and management compatibility between any two breeds of interest. A decision tree is used to classify breed populations based on the level of their breeding programme structure, inbreeding control, genetic improvement strategies, and conservation measures, such as gene banks. The second layer assesses the legislative compliance for collaboration, categorising breeds into specific scenarios defined by EU Breeding legislation (EC 2016/1012), such as the extension of a geographical territory or the existence of multiple approved programmes for the same breed. By integrating these two layers, the framework generates specific guidelines that recommend feasible collaboration strategies, ranging from guidelines in adapting existing herdbooks to the exchange of breeding stock and data. The guidelines also provide technical tools for assessing genetic connectedness between breeds like the Population Differentiation Index (PDI). Ultimately, this framework aims to combine preservation efforts across the EU, enabling endangered breeds to benefit from subpopulations in other countries while ensuring full compliance with EU Breeding legislation (EC 2016/1012).

## Session 1

## Theatre 7

### **Management of Transboundary breeds: The case of Sarakatsaniko sheep breed**

D. Tsiokos<sup>1</sup>, Z. Duche<sup>2</sup>, D. Chatziplis<sup>3</sup>, C. Ligda<sup>4</sup>

<sup>1</sup> Hellenic Agricultural Organization - DIMITRA, RIAS, 58100 Paralimni, Greece, <sup>2</sup> Agricultural Academy, Institute of Animal Science, Pochivka stn, 2232 Kostinbrod, Greece, <sup>3</sup> International Hellenic University, School of Geosciences, Department of Agriculture, Agribiotechnology & Agricultural Products Control Lab, 57400 Sindos, Greece, <sup>4</sup> Hellenic Agricultural Organization - DIMITRA, VRI, 57001 Thessaloniki, Greece

This study is based on the work of the ERFP TF on Transboundary Breeds (TBs). The first phase of the TF focused on the analysis of DAD-IS/EFABIS data providing some new elements on data quality and population management. The analysis showed that TBs cannot be examined exclusively through demographic data and genetic information, as several technical, social and political aspects shape future management opportunities. Thus, a case study approach is recommended. The current work is a preliminary analysis of a TB case study, the Sarakatsaniko (Karakachan) sheep breed, reported in DAD-IS by Bulgaria, Greece, Serbia and Turkey. Population data and information on conservation activities were extracted from DAD-IS. The number of breeding females and of flocks follow similar patterns in Bulgaria and Greece, while in Serbia and Turkey information is scarce. Information on conservation programmes is reported by Bulgaria, Greece and Serbia. In Bulgaria a nucleus flock is established in Rare Breeds Center-Vlahi. Semen from 4 rams is stored in the National genetic reserve, the core part of the Bulgarian gene bank. Genetic studies in Greece show the breed's genetic differentiation from other local breeds in the country. Given the breed's history of selection for resistance to harsh and changing environmental conditions, it would be an ideal model for genetic studies in the context of climate change. The breed is linked to the history of the region and connected with the transhumance practices and movements of populations. The cultural value of the breed is recognized in Bulgaria and Greece, as a common element of local communities, and preliminary actions have been initiated. Conservation activities in both countries provide a solid basis for cooperation to preserve the genetic potential of the breed. This work aims to contribute to the discussion on TBs providing a framework of various components that could be applied to assess similar cases.

### The ARD12 project: Cooperation toward a cross-country breeding scheme involving French and Spanish dairy sheep breeds

C. Pineda-Quiroga<sup>1</sup>, J. M. Astruc<sup>2</sup>, I. Granado-Tajada<sup>1</sup>, A. Zubiri-Gaitán<sup>3</sup>, K. Haugaard<sup>4</sup>, F. Macedo<sup>4</sup>, T. Roozen<sup>4</sup>, E. Ugarte<sup>1</sup>, Z. Vitezica<sup>3</sup>

<sup>1</sup> Neiker-BRTA, Animal Science, Campus Agroalimentario de Arkaute, 01192 Vitoria-Gasteiz, Spain, <sup>2</sup> IDELE, 24 Chem. de Borde Rouge, 31326 Castanet Tolosan, France, <sup>3</sup> INRAE, GenPhySE, 24 Chem. de Borde Rouge, 31326 Castanet Tolosan, France, <sup>4</sup> Interbull Centre, Animal Biosciences-SLU, Box 7023, SE-750 07 Uppsala, Sweden

Latxa and Manech are two genetically related dairy sheep breeds reared in the Basque regions of Spain and France, respectively, each comprising black (LCN and MTN) and red (LCR and MTR) strain. Their breeding schemes have historically been developed separately. Nevertheless, a systematic exchange of germplasm, mainly through artificial insemination doses, has taken place between the breeds over the past 20 years. ARD12 is an ongoing Interreg POCTEFA project (EFA032/01, 2024-2026) aimed at harmonizing genetic tools across breeds to implement an international within-color strain genomic evaluation for milk yield, milk composition, and udder morphology traits, and create a common selection program. At present, the genetic connection relies on 292 animals shared between LCN and MTN, and 1,055 common between LCR and MTR. Genotypic data are also available for 1,075 from MTN and 6,605 from MTR, obtained using low-density (18K) and mid-density (50K) Illumina chips, as well as for 1,982 LCR and 813 LCN animals, obtained using mid-density Illumina and Axiom genotyping arrays. SNP datasets from both technologies were merged based on marker identifiers to create a customized reference map. To optimize the use of genomic data, a collaboration with the Interbull Centre was established, who adapted their International Genotype Exchange Platform (GenoEx) and Interbull Data Exchange Area (IDEA) from cattle to sheep for upload and storage genotypes and pedigree data. Furthermore, they adapted the InterGenomics pipeline to perform genotype quality control, alignment and imputation, delivering genotypes ready for international evaluation. Finally, the European Economic Interest Grouping “ARTAL-DEAN” had also been created in a previous POCTEFA project (ARDI, EFA208/16) as a governance body to support coordinated breeding decisions across both populations and enable the practical implementation of breeding tools.

## Session 1

## Theatre 9

### Manech and Latxa dairy sheep populations: are they becoming closer on a chromosome-by-chromosome basis?

I. Granado-Tajada<sup>1</sup>, E. Ugarte<sup>1</sup>, J. M. Astruc<sup>2</sup>, S. Rodríguez-Ramilo<sup>3</sup>

<sup>1</sup> NEIKER-BRTA, Campus Agroalimentario de Arkaute, 01192 Arcaute, Spain, <sup>2</sup> IDELE, 24 Chem. de Borde Rouge, 31326 Castanet Tolosan, France, <sup>3</sup> INRAE, GenPhySE, 24 Chem. de Borde Rouge, 31326 Castanet Tolosan, France

Latxa Cara Rubia (LCR) and Manech Tête Rousse (MTR) sheep populations, located in northern Spain and southwestern France, respectively, share a long history of genetic connections due to their geographical proximity and the common use of Pyrenean pastures. Exchanges of animals and artificial insemination doses, mainly from MTR to LCR, have been frequent over the past two decades. Both populations have also implemented genomic selection programs supported by extensive genotyping, providing a unique opportunity to study the genomic consequences of these exchanges. Using the LCR flockbook, 661 LCR and 744 MTR genotyped artificial insemination rams were identified and categorized as old (year of birth between 2000–2008) or current (year of birth between 2017–2023). Analyses were carried out between and within populations, considering the evolution over time, on all the autosomal chromosomes, and chromosome-by-chromosome. Genomic coancestry results over time showed an increase in coancestry within each population and is even more pronounced between populations (0.6% MTR, 0.3% LCR, 1.3% between them). Principal component analyses revealed that older LCR rams were genetically more distant from older MTR rams. In contrast, the current LCR rams were closer to contemporary MTR rams, while MTR rams showed little change over time. Ancestry analysis indicated that the LCR population has shifted from 71% to 42% LCR ancestry, moving closer to MTR, whereas MTR individuals have largely retained their original genetic composition (18% mean LCR ancestry). Detailed analysis of Runs of Homozygosity (ROH) at each autosome confirmed that both populations are converging, in agreement with the overall genomic trends. These findings demonstrate that the continuous use of MTR blood in the LCR population has resulted in greater genomic similarity between both populations. Coancestry between LCR and MTR has increased more than within populations, and they share ROH islands. Therefore, these populations are becoming genetically closer, and their evolution is tending toward convergence.

**Digital tools for monitoring and conservation of animal genetic resources: A national good practice example**

T. Flisar<sup>1</sup>, D. Bojkovski<sup>1</sup>, M. Šimon<sup>1</sup>, K. Potočnik<sup>1</sup>

<sup>1</sup> University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

The conservation and sustainable use of Animal Genetic Resources (AnGR) are essential for resilient livestock systems, food security, and the implementation of international biodiversity commitments. Slovenia has established a structured national framework for the management of AnGR, integrating public services, breeding programmes, and digital data infrastructures. Within the project Digitization of Livestock Databases (DigŽiv), funded by the Recovery and Resilience Plan and the Ministry of Agriculture, Forestry and Food, digital solutions were developed to support AnGR monitoring, reporting, and the development of conservation measures. The project aimed to strengthen national capacities for systematic monitoring and evaluation of livestock biodiversity in line with FAO and EU policy objectives, including the Global Plan of Action for AnGR and EU biodiversity strategies. An integrated set of digital tools was developed to support three key functions: monitoring of livestock breeds, assessment of risk status, and management of genetic material conserved ex-situ. These tools provide a clear and transparent overview of livestock breed populations through visual indicators, maps, and summary statistics. A complementary monitoring tool supports expert-based evaluation of population trends and risk status using harmonised criteria, enabling consistent reporting, temporal comparisons, and evidence-based prioritisation of conservation measures. In addition, a traceability solution supports the management of genetic material conserved ex-situ, contributing to the long-term security and sustainable use of national genetic reserves. The Slovenian approach represents a transferable good practice to EU Member States and other countries seeking to strengthen their AnGR information systems. By integrating data management, visual communication, and decision support functions, the developed tools improve the effectiveness of public services, improve policy-relevant reporting, and support the implementation of international and EU-level commitments related to livestock biodiversity conservation.

## Session 1

## Theatre 11

**R\_P\_M: A software to reconstruct pedigrees from molecular markers**

J. Fernández Martín<sup>1</sup>

<sup>1</sup> INIA-CSIC, Animal Breeding, Ctra. Coruña Km 7,5, 28040 Madrid, Spain

In situations where pedigrees cannot be accurately recorded — whether in conservation or in commercial populations under breeding programs — genetic relationships can be directly calculated from molecular markers. However, when molecular information is scarce (as is often the case with non-mainstream breeds), these genetic relationships are not as accurate as those derived from pedigrees. In such cases, a better approach might involve reconstructing genealogies from molecular data, which requires less molecular information. Several methods based on Maximum Likelihood estimation have been developed for pedigree reconstruction, but they suffer from some drawbacks. In this study, a new software tool for pedigree reconstruction (R\_P\_M) is presented. This software implements a method relying on the search for congruent genealogies where the pedigree coancestry matrix between genotyped individuals has the highest correlation with the molecular coancestry matrix. The R\_P\_M software and its underlying algorithm offer high accuracy in pedigree reconstruction, even in situations with scarce molecular information (i.e. few markers with a low level of polymorphism). Additionally, it provides several advantages over currently implemented methods: it is robust against Hardy-Weinberg disequilibrium, independent of the knowledge of real allelic frequencies, capable of incorporating known restrictions (e.g., fixed or banned relationships), and, especially, able to explicitly include virtual individuals to complete the pedigree. Consequently, R\_P\_M serves as a valuable tool for farm/domestic population managers, helping to, among others, estimate genetic relationships between founders of the population, leading to more accurate breeding value estimates and improved management of genetic diversity.

**Comparing Stepwise Discriminant Analyses results across heterogeneous bovine datasets**P. E. Millia<sup>1</sup>, A. Cesarani<sup>1,2</sup>, N. P. P. Macciotta<sup>1</sup>, C. Dimauro<sup>1</sup><sup>1</sup> University of Sassari, Dipartimento di Agraria, Viale Italia 39a, 07100 Sassari, Italy, <sup>2</sup> University of Georgia, Department of Animal and Dairy Science, 425 River Rd, 30602 Athens, United States

In a previous study, three Sardinian cattle breeds—Sarda (SAR), Sardo-Bruna (SB), and Sardo-Modicana (SM)—were investigated to evaluate the persistence of SAR genomic background in SB and SM, derived from crossbreeding with Brown Swiss and Modicana bulls. A total of 141 animals were genotyped using the Illumina 50K SNP BeadChip (ARS-UCD1.2). After quality control, 35057 SNPs were retained. Genomic regions potentially conserved among breeds were first identified using a threshold based on Wright's fixation index (FST) from SAR vs. SB and SAR vs SM comparisons. Selected markers were then analyzed using Stepwise Discriminant Analysis (SDA) with an inverse approach: the most discriminant markers were progressively removed until in the subsequent discriminant analysis developed with the remaining markers, the groups were not significantly separated. This procedure identified 74 and 79 SNPs in the SAR vs SB and SAR vs SM comparisons, respectively, distributed across several autosomes. The same framework was applied to a new dataset of 271 animals genotyped with the Affymetrix 100K platform and mapped to the ARS-UCD2.0 assembly. After quality control, 86225 SNPs were retained, of which 38060 (SAR vs SB) and 36736 (SAR vs SM) exceeded the FST threshold. SDA identified 150 markers with no discriminant power across both comparisons. Due to differences between datasets, direct marker-by-marker comparisons was not always feasible; therefore, analyses focused on genomic regions rather than individual SNPs assessing concordance by matching regions within  $\pm 250$  kb windows around each candidate position. Overlapping regions were found on BTA11 (4.66–4.98 Mb) for SAR vs SB and on BTA4 (36.91–37.22 Mb) and BTA8 (44.23–44.37 Mb) for SAR vs SM. In addition to confirming markers on the same autosomes reported in the previous study, additional candidate regions were identified on other chromosomes. These findings demonstrate that the region-based SDA signals are reproducible across heterogeneous datasets and that a region-focused approach provides a robust framework for comparing studies generated using different platforms and genome assemblies.

## Session 1

## Theatre 13

**Supporting genetic management in European dromedary farming using genomic tools**S. Bruno<sup>1</sup>, C. I. Pastrana<sup>2,3</sup>, J. Müller<sup>4</sup>, A. Mehrotra<sup>4</sup>, M. G. Molina<sup>1,5</sup>, G. Bramante<sup>1</sup>, E. Ciani<sup>1</sup><sup>1</sup> University of Bari, DBBA, Via Orabona 4, 70100 Bari, Italy, <sup>2</sup> Alameda del Obispo, IFAPA, Av. Menéndez Pidal, S/N, 14001 Cordova, Spain, <sup>3</sup> Cordoba University, Dept. of Genetics, Campus Rabanales, 14001 Cordova, Spain, <sup>4</sup> Generatio GmbH, Blumenstraße 16, 69115 Heidelberg, Germany, <sup>5</sup> FCA-UNC, Ciudad Universitaria, Dept. of Animal Production, Avenida Haya de la Torre, 5000 Córdoba, Argentina

Dromedary camels are increasingly being reared in Europe, underscoring need for structured genetic management in a sector that has thus far developed with limited pedigree control. Within the Illumina® Agricultural Greater Good Initiative and the EU-funded CAMEL-SHIELD project, we (i) genetically characterized European dromedaries using the recently developed Illumina® Camel SNP60K genotyping array and (ii) developed and validated SNP panels for parentage verification in *Camelus dromedarius*. A total of 332 camels from 16 herds were sampled across five European countries (Spain, France, Belgium, the Netherlands, and Cyprus). Overall and within-herd classical genetic parameters were estimated to monitor genetic diversity and to evaluate the effectiveness of current management practices, particularly in larger herds. To investigate genetic relationships and infer the most likely origins of European stocks, these genotypes were combined with previously generated datasets including extra-European dromedaries. For parentage verification, two SNP panels of approximately 100 loci each were derived from a globally representative dataset of 374 dromedaries genotyped with the Camel SNP60K array. In both cases, loci were selected primarily based on minor allele frequency. Subset A included the most informative SNPs from the full quality-controlled Camel SNP60K dataset (Probability of Identity – PI =  $1.0E-42$ ), while Subset B was derived from 6k SNPs shared with the Axiom™ Camelids Genotyping Array (PI analysis ongoing) to ensure platforms interoperability. Here, the results of the above studies are discussed, demonstrating how genomic tools can support the transition of European dromedary farming from a poorly structured niche sector to a modern production system with effective diversity monitoring, reliable parentage verification, and the establishment of formal pedigree registries.

**Tracing worldwide turkey expansion through biometrics: from Central America to the Mediterranean**

J. I. Salgado Pardo<sup>1</sup>, A. González Ariza<sup>2</sup>, J. V. Delgado Bermejo<sup>1</sup>, C. Rivas López<sup>1</sup>, J. M. León Jurado<sup>2</sup>, S. P. Marelli<sup>3</sup>, S. Cerolini<sup>3</sup>, L. Zaniboni<sup>3</sup>, A. Vargas Cornejo<sup>4</sup>, M. Winter Dendena<sup>4</sup>, M. Barrientos Morales<sup>5</sup>, P. Cervantes Acosta<sup>5</sup>, A. Hernández Beltrán<sup>5</sup>, M. E. Camacho Vallejo<sup>6</sup>

<sup>1</sup> University of Córdoba, Genetics Department, C5 Gregor Mendel building, Rabanales University Campus, 14071 Córdoba, Spain, <sup>2</sup> Diputación of Córdoba, Agropecuaria Provincial Centre, N-IV road, Km.396, 14071 Córdoba, Spain, <sup>3</sup> University of Milan, Department of Veterinary Medicine, Via Alessandro Volta, 26900 Lodi, Italy, <sup>4</sup> Polytechnic University of Francisco I. Madero, Domicilio Conocido SN, 42660 Hidalgo, Mexico, <sup>5</sup> University of Veracruz, School of Veterinary Medicine and Animal Science, Circunvalación s/n, Veracruzana, 91710 Veracruz, Mexico, <sup>6</sup> Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Department of Agriculture and Ecological Husbandry, IFAPA Alameda del Obispo, 14004 Córdoba, Spain

The present study aims to describe the relationship between Central American and Mediterranean indigenous turkeys based on their biometrics. To this end, 255 adult turkeys were measured, comprising Spanish (Andalusian), Italian (Brianzolo and Nero d'Italia) and Mexican (from the states of Hidalgo and Veracruz) landraces. The birds underwent a protocol including body measurements, biometric indices and qualitative variables. A discriminant canonical analysis was carried out for each sex separately using the genotype as categories, and the different biometric items as the independent variables. Both the Pillai's trace criterion and the Wilk's Lambda test were significant, allowing the discriminant approach. The observations were represented graphically, and the similarities between groups were explained using the Euclidean distance between each group's centroid. Although there was a slightly different clustering pattern across sexes, the Andalusian and Hidalgo turkeys were always closely grouped. However, the clustering pattern of the females was more closely aligned with the descriptions of molecular genetic studies, placing the Spanish population between the Mexican Hidalgo and Italian landraces.

**Population, Production and Social Determinants of Sustainability in Local Cattle Breeds: Examples from Croatia**

A. Ivanković<sup>1</sup>, Z. Ivkić<sup>2</sup>, M. Konjačić<sup>1</sup>, N. Kelava Ugarković<sup>1</sup>

<sup>1</sup> University of Zagreb Faculty of Agriculture, Department of Animal Science and Technology, Svetošimunska cesta 25, 10000 Zagreb, Croatia, <sup>2</sup> Croatian Agency for Agriculture and Food, Ulica kardinala Alojzija Stepinca 17, 31000 Osijek, Croatia

The long-term sustainability of local cattle breeds is shaped by population, production, and social factors, as well as by stakeholder coordination. This study aimed to assess the significance of these indicators for the success of maintenance and reaffirmation of local cattle breeds in Croatia (Buša, Istrian cattle and Slavonian-Syrmian Podolian cattle). The analysis included long-term structural population indicators (age and sex structure of the population, replacement rate, age of breeders, etc.), pedigree records, meat productivity traits, breeders' organizational capacity and market integration, policy support at national, regional and local levels, social perception of breed importance and ecological footprint. Pedigree data were analysed using ENDOG v4.8 software, meat productivity was assessed at the slaughter line, while qualitative indicators were quantified using a point-based scoring system (scale 1 to 9) derived from document analysis, media presence, market activities and promotional events. Indicators were standardized and weighted to produce an overall sustainability score. Despite having the largest population (5,263 animals; 437 breeders), Buša has lower sustainability compared to Istrian cattle (1,539 animals; 172 breeders), primarily due to weak market integration and insufficient regional and local support for infrastructure development. In contrast, Istrian cattle benefit from strong regional policy support linked to economic valorisation through gastronomy, reflected in higher daily weight gains of calves (661 vs. 457 g) and a higher proportion of carcasses classified as 'R' and 'O' (94.3%). The Slavonian-Syrmian Podolian cattle population has an unfavourable inbreeding level (5.41%) compared to Istrian cattle (2.98%) and Buša (2.43%). Overall, the results demonstrate that regional and local governance plays a decisive role in achieving the long-term self-sustainability of endangered local cattle breeds.

**Validation of Pedigree Accuracy in Istrian Sheep Using SNP Markers**

M. Špehar<sup>1</sup>, D. Ceranac<sup>1</sup>, J. Ramljak<sup>2</sup>, Z. Barac<sup>3</sup>, V. Držaić<sup>2</sup>, B. Mioč<sup>2</sup>, D. Jurković<sup>1</sup>, Z. Ivkić<sup>1</sup>, I. Pocrnic<sup>4</sup>, A. Kasap<sup>2</sup>

<sup>1</sup> Croatian Agency for Agriculture and Food, Svetošimunska cesta 25, 10000 Zagreb, Croatia, <sup>2</sup> Croatian Association of sheep and goat breeders, Jarnovičeva 17c, 10000 Zagreb, Croatia, <sup>3</sup> University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia, <sup>4</sup> University of Edinburgh, Roslin Institute, EH25, 9RG Edinburgh, United Kingdom

This study focused on the critical analysis of pedigree accuracy within the Istrian sheep population, an indigenous breed reared in semi-extensive farming systems. Such systems often present significant challenges for precise field data recording. The objective of the study was to perform pedigree correction using genomic information obtained via a medium-density SNP chip, establishing a reliable foundation for future breeding and selection programs aimed at enhancing milk production. A total of 1554 individuals were included in the genomic analysis. The analysis was performed using SeekParentsF90, applying a 1.0% Mendelian conflict threshold for pedigree exclusion and a rigorous 0.5% threshold for new assignments to minimize false-positive discoveries. The results revealed notable discrepancies between the official herd-book records and actual genetic relatedness. The genomic validation successfully re-established the correct biological relationships for 43 sires and 143 dams, previously unrecorded or incorrectly documented in the pedigree. These findings support the hypothesis that semi-extensive rearing conditions, despite the best efforts of breeders, carry a high risk of parental misidentification at the time of lambing. This correction significantly enhances the integrity of the database, providing the necessary accuracy for estimating genetic parameters and implementing effective selection strategies for dairy traits. Finally, this research confirms that the application of genomic tools in indigenous sheep populations is a mandatory prerequisite for the success of selection programs, whether they are oriented towards genetic gain or the preservation of genetic variability.

**Population structure and genetic relationships among Mediterranean island cattle breeds**

P. E. Milia<sup>1</sup>, A. Cesarani<sup>1,2</sup>, N. P. P. Macciotta<sup>1</sup>, C. Dimauro<sup>1</sup>

<sup>1</sup> University of Sassari, Dipartimento di Agraria, Viale Italia 39a, 07100 Sassari, Italy, <sup>2</sup> University of Georgia, 2Department of Animal and Dairy Science, 425 River Rd, 30602 Athens, United States

Sarda (SAR) and Corsa (COR) cattle are autochthonous breeds from Sardinia and Corsica. These breeds were historically described as closely related and phenotypically similar to other Mediterranean island breeds, including Mallorquina (MAL) and Menorquina (MEN). The aim of this study was to investigate shared ancestry and historical gene flow among 4 Mediterranean island cattle breeds. A total of 157 genotyped animals (64 SAR, 33 COR, 30 MAL, and 30 MEN), with 31704 common SNPs, were analyzed. Population structure was investigated using principal component analysis (PCA) based on the genomic relationship matrix, pairwise Wright's fixation index (FST), and admixture analysis (K = 2–5). Runs of homozygosity (ROH) were used to infer patterns of autozygosity and demographic history. PCA revealed a close clustering of SAR and COR, indicating strong genetic affinity, while MEN showed an intermediate position and MAL formed a clearly distinct cluster along the first principal component. Admixture analysis supported these results, showing shared ancestral components between SAR and COR, partial contribution in MEN, and a distinct ancestral profile for MAL across all tested K values. Pairwise FST estimates confirmed these patterns, with the lowest genetic differentiation between SAR and COR (FST = 0.034) and the highest values involving MAL. ROH analysis identified a replicated homozygous region on BTA13 shared by individuals from all four breeds. This region harbored genes associated with adaptation to dry environments, carcass and meat quality, fertility, reproduction, and stayability (including PLCB1, PLCB4, JAG1, and TASP1). Overall, the identification of shared genomic features supports a close genetic relationship among Mediterranean island cattle breeds and reflects their common evolutionary history. These results highlight the value of genomic characterization of local breeds to support conservation and sustainable management strategies.

### Genome-wide SNP profiling of Greek insular sheep breeds to reveal population structure and diversity

I. Apostolakos<sup>1</sup>, V. Tsartsianidou<sup>2</sup>, M. A. Karatzia<sup>3</sup>, E. Boukouvala<sup>1</sup>, A. Triantafyllidis<sup>2</sup>, L. V. Ekateriniadou<sup>1</sup>

<sup>1</sup> Veterinary Research Institute, Hellenic Agricultural Organization, Demeter, Elgo Demeter Campus, 57001 Thessaloniki, Greece, <sup>2</sup> School of Biology, Aristotle University of Thessaloniki, Department of Genetics, Development & Molecular Biology, University Campus, 54124 Thessaloniki, Greece, <sup>3</sup> Research Institute of Animal Science, Hellenic Agricultural Organization, Demeter, Paralimni Giannitswn, 58100 Pella, Greece

Sheep farming is a cornerstone of agricultural activity in the Greek islands, where long-term geographic isolation and environmental heterogeneity have shaped distinct production systems. Insular sheep populations exhibit substantial genetic differentiation, reflecting marked phenotypic variation in productive, reproductive, and adaptive traits, hence they play a critical role as reservoirs of resilience. The ENIPRO project aims to characterize genotypic and phenotypic variation in island sheep populations to elucidate adaptation mechanisms and support sustainable conservation and utilization of these uniquely adapted genetic resources. The study will focus on the genome-wide characterization of population structure, diversity and admixture of representative insular sheep populations. Blood samples (n=30-50/breed) have been collected from seven insular breeds (Kefallinias, Zakynthos, Karystou, Kymi, Lesvos, Asterousia, Aegean) for genotyping using the Illumina OvineSNP50 BeadChip. Principal component analysis and model-based clustering will be carried out to assess the genetic relationships among populations, define genetic clusters and infer individual ancestries. Genetic differentiation between island populations will be evaluated using pairwise fixation indices. Heterozygosity and inbreeding coefficients will be estimated to assess genetic diversity across and within breeds. This analysis will provide valuable insights into the genetic diversity reservoirs in the Mediterranean changing environment, supporting the design of targeted conservation programs, to preserve the genetic identity of local breeds. This research is implemented under the National Recovery and Resilience Plan 'Greece 2.0', funded by the European Union – NextGenerationEU.

### The impact of grazing small ruminants on limiting the expansion of *Solidago gigantea* in Poland

M. Pasternak-Choros<sup>1</sup>, A. Kawęcka<sup>1</sup>, M. Puchała<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, Department of Sheep and Goat Breeding, Krakowska 1, 32-083 Balice n. Kraków, Poland

Small ruminant grazing can benefit valuable natural landscapes and help preserve the unique character of ecosystems and the biodiversity of wild species. It can also be used to limit the spread of invasive plants, like *Solidago gigantea*. These cause economic damage and can negatively impact the environment by transforming natural habitats and displacing native species through competition or by limiting their food supply. None of the chemical and mechanical control methods are entirely effective, so an attempt was made to utilize native sheep and goat breed grazing for this purpose in southern Poland. The *Solidago gigantea* pasture was divided into three sections: the first section was grazed by 6 Świniarka sheep, the second section by 6 Carpathian goats, and the third section was mown. After each of two grazing sessions, the uneaten grass was removed, and the pasture was then left for the sward to regrow. Botanical inventory involved assessing the presence of *Solidago gigantea* individuals and non-target plant species, providing an estimate of their area coverage (%). Initial *Solidago gigantea* cover in the experimental fields averaged approximately 32.6%. After the first stage, it dropped to an average of 12.5%. In the sheep-grazed section, it decreased to 1.5%, and by the end of the second stage, it was close to zero (0.5-1%). Goat grazing gradually reduced the invasive population (8-12%), and ultimately, in this section, it was almost completely reduced by the final stage of the experiment. Mowing reduced the *Solidago gigantea* occurrence by 88%. Grazing effectively reduced the layer of residual felt and contributed to a significant increase in the plant biodiversity index. The largest increase in species numbers was observed in the sheep-grazed section, from 10 to 14 species. The results from monitoring the effects of grazing in reducing the occurrence of *Solidago gigantea* showed that the fastest method to obtain satisfactory results and with the greatest impact on plant biodiversity increase was sheep grazing, followed by goat grazing. The most important issue observed was the sustainability of the treatments, which should be continued for several consecutive seasons.

**The quality of milk from Carpathian goats grazing on pastures containing *Solidago gigantea***M. Pasternak-Choros<sup>1</sup>, A. Kawęcka<sup>1</sup>, M. Puchala<sup>1</sup><sup>1</sup> National Research Institute of Animal Production, Department of Sheep and Goat Breeding, Krakowska 1, 32-083 Balice n. Kraków, Poland

Goat milk is considered healthy and nutritious. Goat milk composition depends on the season, lactation stage and diet. Goats, as small ruminants, are kept on pastures for most of the grazing season, where pasture plants are their main source of nutrition. The aim of this study was to analyse the composition of milk from goats grazing on pastures overgrown with *Solidago gigantea*. Grazing was conducted to limit the spread of this invasive plant. Six Carpathian goats were grazed on pasture overgrown with *Solidago gigantea*. Grazing consisted of two 6-week sessions: May/June and July/August. The control group consisted of six goats grazing on pasture without *Solidago gigantea*. Pooled milk samples were collected on three separate dates in May, June, and August. The milk was assessed for basic composition, amino acids, minerals, protein, and phenol content. Milk from goats grazing on a goldenrod pasture contained significantly more dry matter, protein, and fat (12.82%, 4.34%, 3.59%) than milk from goats in the control group (12.24%, 3.91%, 3.36%). Milk from goats in the experimental group was also characterized by significantly higher acidity and higher content of serine, glycine, histidine, threonine, alanine, cysteine, tyrosine, valine, and phenylalanine. The content of Mn, P, and Zn was higher in the milk of goats in the control group, while the experimental group had a significantly higher content of Na. Milk protein electrophoresis showed that milk from goats in the experimental group contained significantly more immunoglobulins (6.78%) than milk from goats in the control group (3.97%). It was also characterized by a lower AS2 content (9.05 VS 10.49%) and a higher A-LA level (7.75 VS 6.43%). The content of total phenols was significantly higher in the milk of goats grazing on the goldenrod pasture (34.9 VS 31.67 mg/l). The composition of milk from the experimental and control goats differed significantly across many parameters. Goats grazing on pasture containing goldenrod had significantly increased milk content of both essential nutrients and most amino acids. Their milk also exhibited increased content of A-LA, immunoglobulins, and phenols, which are valuable for the human health.

## Session 1

## Poster 21

**Genetic resistance to scrapie in Ile de France breed in Bulgaria**Z. Ducheve<sup>1</sup>, M. Bozhilova-Sakova<sup>1</sup>, T. Ivanova<sup>1</sup>, E. Achkakanova<sup>1</sup><sup>1</sup> Institute of Animal Science, Agricultural Academy, Pochivka stn, 2232 Kostinbrod, Bulgaria

Ile de France is one of the transboundary breeds introduced in Bulgaria, with current population size of around 11000 sheep. Animals from France are continuously imported to enhance some of the local flocks. To assess the genetic diversity of the PRNP gene associated with susceptibility to scrapie in sheep, 94 animals were genotyped. Genotyping was performed by PCR-amplification and Sanger sequencing of the exon 3 open reading frame. The animals were selected from various flocks, based on their genetic relationship. Three haplotypes were identified – ARR, ARQ and VRQ. The ARR haplotype, which is strongly associated with resistance to classical scrapie, was observed with a frequency of 0.96, a significant increase since the previous study by another team in Bulgaria. The unfavorable VRQ haplotype, which is considered linked to susceptibility to the disease, was found only in this study in two animals. Three genotypes were identified: ARR/ARR, ARR/ARQ, and ARR/VRQ, with ARR/ARR being the most frequent (0.93). This confirms the position of Ile de France as the breed with the highest frequencies of ARR haplotype and ARR/ARR genotype in the country, followed by another transboundary breed – Assaf. In the native Bulgarian breeds, the ARR/ARR genotype has been reported in frequencies lower than 0.25. Of the studied animals, 98% fall in the groups with lowest risk of infection, providing evidence of a high degree of genetic resistance to classical scrapie. The results indicate that the mild selection measures applied by the breeding association are successful in increasing favourable haplotypes, indicating effective implementation of scrapie-resistance breeding strategies.

### Genome-wide comparisons between whole-genome sequencing and high-density SNP data in Sicilian cattle breeds

S. Tumino<sup>1,4</sup>, G. Chessari<sup>1</sup>, A. Antoci<sup>1</sup>, M. Carlentini<sup>1</sup>, S. Mastrangelo<sup>2,4</sup>, A. Cesarani<sup>3,4</sup>, S. Bordonaro<sup>1,4</sup>, D. Marletta<sup>1,4</sup>, A. Criscione<sup>1</sup>

<sup>1</sup> University of Catania, via Santa Sofia, 95123 Catania, Italy, <sup>2</sup> University of Palermo, viale delle scienze, 90128 Palermo, Italy, <sup>3</sup> University of Sassari, viale Italia, 07100 Sassari, Italy, <sup>4</sup> Centro Interuniversitario A.SI.ZO.CA.CLIstituto AS, via santa Sofia, 95123 Catania, Italy

Cinisara (CIN) and Modicana (MOD) are cattle breeds native to Sicily and represent an important component of Mediterranean livestock biodiversity. In addition to their historical and cultural relevance, they are well adapted to environmental stressors such as heat, drought, and scarce feed or water, conditions that are intensifying under climate change. Understanding their genetic structure and diversity is essential for conservation, sustainable management, and identifying genomic regions involved in adaptation and resilience. Previous analyses of 76 CIN and 58 MOD cattle genotyped with the Illumina BovineHD BeadChip identified recurrent runs of homozygosity (ROH) associated with fertility, production, growth, meat quality, and disease resistance, while heterozygosity-rich regions (HRR) were linked to immunity and fatty acid composition. In this study, we compared ROH and HRR patterns derived from WGS, imputed, and HD genotyping data to assess the added value of imputation in population genetic analyses. WGS data were generated from 15 CIN and 15 MOD individuals at  $\sim 22\times$  coverage. Reads were processed following GATK Best Practices and aligned to the ARS-UCD1.3 reference genome. Variant calling was performed using HaplotypeCaller in GVCF mode, followed by hard filtering and Variant Quality Score Recalibration using two SNP array datasets as truth panels. The final dataset included over 20 million variants with an  $\sim 80\%$  PASS rate. After phasing of genotypes, this dataset was used as a reference panel for imputation. This approach enables a more accurate characterization of genetic relationships and homozygosity/heterozygosity patterns. Also, it evaluates whether imputation from HD genotypes can reliably approximate WGS-based results as a cost-effective strategy for livestock biodiversity studies. The research was funded by “Gen.AI.Tec.” Linea 1-PIAno di inCentivi per la Ricerca di Ateneo 2024/2026.

## Session 1

## Poster 23

### Frequency of the $\beta$ -casein A2 gene in native cattle breeds: Polish Red and Polish Black-and-White

I. Radkowska<sup>1</sup>, K. Ropka-Molik<sup>2</sup>, D. Rubiś<sup>3</sup>

<sup>1</sup> National Research Institute of Animal Production, Department of Cattle Breeding, Sarego 2, 31-047 Krakow, Poland, <sup>2</sup> National Research Institute of Animal Production, Department of Animal Molecular Biology, Sarego 2, 31-047 Krakow, Poland, <sup>3</sup> National Research Institute of Animal Production, Department of Animal Molecular Biology, Sarego 2, 31-047 Krakow, Poland

The occurrence of  $\beta$ -casein variants in cow's milk is genetically determined, and their identification constitutes an important element of breeding programs aimed at improving milk quality. The aim of this study was to assess the frequency of genes encoding  $\beta$ -casein, with particular emphasis on the A2 variant, in Polish Red and Polish Black-and-White cows. Genetic material was collected from Polish Red cows kept on individual farms and from Polish Black-and-White cows maintained at the Experimental Station of the National Research Institute of Animal Production in Chorzew. The DNA samples were isolated from hair follicles. Genotyping of  $\beta$ -casein variants was performed using the PCR Allelic Discrimination qPCR method. Analysis of genotypes in 273 Polish Red cows showed that 20% of animals were A2A2 homozygotes, 49% were A1A2 heterozygotes, and approximately 31% were A1A1 homozygotes. The frequency of genotypes varied depending on herd size. The highest proportion of A2A2 homozygotes (24%) was observed in herds exceeding 100 cows, whereas the highest proportion of A1A1 homozygotes (46%) occurred in the smallest herds (10–20 animals). In the Polish Black-and-White population, a clear predominance of the A1 allele was observed. The distribution of genotypes was as follows: A1A2 – 48%, A1A1 – 47%, and A2A2 – 5%, corresponding to allele frequencies of 0.71 for A1 and 0.29 for A2. Deviations from Hardy–Weinberg equilibrium were detected, characterized by an excess of heterozygotes, which may indicate the influence of breeding selection and population structure. The results indicate that the current potential for producing exclusively A2 milk in the studied population is limited; however, the presence of the A2 allele provides an opportunity to increase its frequency through targeted breeding strategies.

### Assessment of leptin receptor genotypes in Majorcan Black pigs

P. Pascual<sup>1,2</sup>, J. Jaume<sup>3</sup>, J. Estany<sup>1</sup>

<sup>1</sup> University of Lleida, Animal Science, Rovira Roure 191, 25198 Lleida, Spain, <sup>2</sup> Association of Majorcan Black Pig Breeders, Aigua, 4, 07510 Sineu, Spain, <sup>3</sup> Institute for Agri-food Research and Training, Eusebi Estada, 145, 07009 Palma, Spain

The Majorcan Black pig is a fatty, native breed of Mallorca, typically reared under extensive or semi-extensive farming systems. It is valued primarily for the production of traditional suckling pig (porcella) during island festivities and for the manufacture of sobrassada de Mallorca, a traditional spreadable pork sausage seasoned with paprika and registered as an EU Protected Geographical Indication product. Nevertheless, the breed is limited by relatively poor production and reproductive efficiency. It has been reported that the missense polymorphism rs709596309 C>T in the leptin receptor gene (LEPR) segregates in the breed. In previous studies with premium Duroc pigs, we showed that individuals homozygous for the T allele, although exhibiting higher marbling, have lower maternal ability, are fatter, and less feed-efficient. Here, we present the first results of an experiment conducted to investigate the (1) the frequency of the T allele in marketed Majorcan Black pigs and (2) its effects on growth performance, fatness, and feed efficiency. A secondary objective is to lay the groundwork for the establishment of systematic data collection and a DNA bank to support future studies. At present, longitudinal data on body weight, backfat thickness, and feed intake are being collected throughout the fattening period in 148 commercial Majorcan Black pigs fattened on a single farm. Results obtained to date indicate that the T allele segregates at an intermediate allele frequency (0.46) in marketed Majorcan Black pigs and, as expected, appears to be associated with increased growth and fatness. At 165 days of age, TT pigs showed +9.4 kg body weight ( $P < 0.05$ ) and +2.7 mm backfat thickness ( $P < 0.10$ ) compared with CC pigs. Based on this evidence, the impact of the LEPR gene on selection and conservation strategies is currently being evaluated. Data collection in local breeds is often challenging. Beyond its direct implications, studying a major gene like LEPR has the potential to encourage associations and farmers to adopt more consistent recording and monitoring practices, positively impacting breeding and management programs.

### Session 1

### Poster 25

### Estimation of Breeding Values and genetic parameters for Milk Yield in Patch-faced Maritza Sheep Breed

H. Grosu<sup>1</sup>, P. Zhelyazkova<sup>2</sup>, D. Dimov<sup>3</sup>, C. R. Cernitu<sup>4</sup>

<sup>1</sup> University of Agronomic Sciences and Veterinary Medicine, Faculty of Animal Productions Engineering and Management, Bulevardul Mărăști 59, Sector 1, 011464 Bucharest, Romania, <sup>2</sup> Breeding Association of Maritza Sheep Breeds, 69 Konstantin Velichkov str., 4000 Plovdiv, Bulgaria, <sup>3</sup> Agricultural University, Faculty of Agronomy, 12, Mendeleev str., 4000 Plovdiv, Bulgaria, <sup>4</sup> University of Agriculture and Veterinary Medicine, Faculty of Animal Productions Engineering and Management, Bulevardul Mărăști 59, Sector 1, 011464 Bucharest, Romania

The aim of this research was to estimate the breeding values and the genetic parameters for milk yield in a nucleus flock belonging to the Patch-Faced Maritza sheep breed. Data analysis was performed using the BLUP (Best Linear Unbiased Prediction) methodology – animal model with repeatability. The sommer package in the R programming language (specifically the mmes function) was utilized. The statistical model included fixed effects such as lambing year (FY – 18 levels), ewe age (Age – 9 levels), and lactation period (84 levels). Random effects consisted of the additive genetic value (animal) and the permanent environmental effect (pe). The pedigree structure comprised a total of 504 animals, including 35 sires and 200 dams. A total of 643 performance records were analyzed, originating from 351 ewes. The estimated genetic parameters indicate a heritability for milk yield of  $h^2 = 0.262 \pm 0.024$  and a repeatability of  $0.525 \pm 0.049$ . These values fall within the ranges reported by other authors for various sheep populations. The intermediate heritability value demonstrates that selection for this trait generates moderate genetic progress within the investigated population. The significant difference between heritability and repeatability underscores the importance of optimizing farm management factors, given the major impact of the permanent environment on productive performance. The use of modern algorithms from the sommer package allowed for an accurate genetic evaluation of all 504 animals, providing a rigorous basis for the ranking and selection of breeding stock. Keywords: milk yield, heritability, repeatability, animal model, sommer, BLUP.

**A regional swine semen production center to support small and medium pig farms in Sardinia**

F. Chessa<sup>1</sup>, I. Cossu<sup>1</sup>, F. Melis<sup>1</sup>, G. L. Belloi<sup>2</sup>, C. Nassif<sup>3</sup>, M. Gallus<sup>1</sup>, A. Manunta<sup>3</sup>, G. Pozzato<sup>4</sup>, G. Epifani<sup>1</sup>, M. Datte-  
na<sup>1</sup>, L. Mara<sup>1</sup>

<sup>1</sup> Agris, Animal Science, Agricultural Research, SS 291 KM 18,600, 07100 Sassari, Italy, <sup>2</sup> Laore, Via Sandro Pertini, 08100 Nuoro, Italy, <sup>3</sup> AARS, Piazzale Segni 1, 07100 Sassari, Italy, <sup>4</sup> ANAS, Via d'Este 6, 42044 Gualt-  
ieri Reggio Emilia, Italy

The C.R.S.A. (SuinAgris Swine Reproduction Center) is the first regional facility in Sardinia dedicated to the production, preservation, and distribution of refrigerated and frozen swine semen. Established through regional funding programs in 2018 and 2021, the center aims to support small- and medium-sized pig farms by improving reproductive efficiency and genetic quality while reducing disease transmission risks through centralized reproductive management. The center is located at the Bonassai experimental farm (Sassari, Italy). During the pilot phase, activities were carried out in collaboration with the Istituto Zooprofilattico Sperimentale of Sardinia, Laore Sardegna, Agris Sardegna, the University of Sassari, and ANAS, providing sanitary control, farmer training, technological support, and research on semen quality, respectively. Current semen distribution across the island is managed in collaboration with AARS. Activities include semen collection and processing (fresh and frozen), qualitative assessment using Computer Assisted Sperm Analysis (CASA), artificial insemination trials, and training and certification courses for farmers. Semen is obtained from terminal boars of Large White, Duroc, and Italian Landrace breeds registered in the ANAS Herd Book. Semen doses (80 mL,  $2.5 \times 10^9$  spermatozoa) can be stored for up to 10 days at 15 °C and are subjected to weekly quality controls evaluating concentration, total and progressive motility, and sperm morphology. Since its opening on 11 March 2024, 98 users have registered on the SuinAgris online platform and 900 semen doses have been sold. Preliminary results show a steady increase in weekly demand, confirming the strategic role of the C.R.S.A. in enhancing modernization and biosecurity of swine production systems in Sardinia

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**Polymorphism of  $\kappa$ -casein (CSN3) in selected cattle breeds: Polish Black-and-White, Polish Red-and-White, Polish Red, and Simmental**

M. Skarwecka<sup>1</sup>, I. Radkowska<sup>1</sup>, K. Żukowski<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, Department of Cattle Breeding, 2 Sarego Street, 31-047  
Krakow, Poland

Cow's milk contains four main casein fractions –  $\alpha$ s1,  $\alpha$ s2,  $\beta$ , and  $\kappa$  – which form casein micelles and thereby determine the stability of the milk protein system.  $\kappa$ -casein is of particular technological importance, and its genetic polymorphism (alleles A, B, and E) significantly influences the suitability of milk for processing. Milk from cows with the BB genotype is characterized by a shorter coagulation time, a stronger and more elastic curd, an 8–12% higher cheese yield, better protein recovery, and greater stability during processing. The aim of the study was to assess the frequency of  $\kappa$ -casein (CSN3) genotypes among four cattle breeds: Polish Black-and-White, Polish Red-and-White, Polish Red, and Simmental. A total of 8874 individuals (7872 cows and 1002 bulls) were analyzed. The results demonstrated significant breed-related differences in CSN3 genotype distribution. In the Polish Black-and-White cattle population, genotype AA predominated (71%), genotype AB accounted for 25%, BB for 2.6%, and genotypes AE and BE together comprised 1.4%, indicating a very low frequency of allele B. The Polish Red-and-White breed displayed the greatest genotypic diversity: genotype AB was most frequent (47%), followed by AA (28%), and genotype BB accounted for 17.5%. In the Polish Red cattle population, genotypes AA and AB were observed at equal frequencies (44% each), with BB at 10%. The largest population, Simmental cattle, was characterized by a predominance of genotype AA (57%), a high proportion of AB (37%), and a low frequency of BB (5.5%). Allele A predominated in all breeds. However, the highest frequency of genotypes containing the technologically favorable allele B was observed in the Polish Red-and-White and Polish Red cattle. These findings confirm the rationale for using  $\kappa$ -casein gene polymorphism as a selection tool in breeding programs aimed at improving the technological quality of milk.

**Building a National Framework for Animal Genetic Resources Conservation: Strategic and Policy Perspectives from Poland**E. Sosin<sup>1</sup>, A. Chelmińska<sup>1</sup>*<sup>1</sup> National Research Institute of Animal Production, Department of Farm Animal Biodiversity Conservation and Horse Breeding, Ul. Krakowska 1, 32-083 Balice, Poland*

Structural, economic and environmental transformations observed in the livestock sector in recent years have significantly increased the policy relevance of animal genetic resource (AnGR) conservation. Genetic diversity is no longer perceived solely as a conservation objective, but increasingly as a strategic component of agricultural resilience, food security and crisis preparedness. In Poland, these developments have necessitated an update of the National Strategy for the Sustainable Use and Conservation of Animal Genetic Resources, previously implemented for the period 2013–2025. The aim is to present the rationale, policy context and principal directions of the updated National Strategy for 2026–2035. The updated Strategy remains aligned with the European Animal Genetic Resources Strategy and the FAO Global Plan of Action. Particular emphasis is placed on strengthening population characterisation and monitoring, advancing digitalisation and integrated data systems, promoting sustainable use of native breeds through “conservation by use”, and systematically developing ex situ conservation as a complementary measure to in situ programmes. A central policy innovation of the revised Strategy is the reinforcement of ex situ conservation through the National Bank of Biological Material, envisaged as the core of a coordinated national gene bank network. The Strategy also highlights the importance of regional approaches, development of added value for native breeds, certification schemes, and short supply chains as mechanisms supporting economically viable conservation. Effective governance, stakeholder coordination, capacity building and public awareness are identified as critical prerequisites for successful implementation. Overall, the updated National Strategy positions AnGR conservation within a broader policy framework encompassing agricultural sustainability, climate adaptation and long-term food security, strengthening the role of native breeds as a strategic resource for the resilience of the livestock sector.

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**Comparison of the processes of breeding native goats**J. Sikora<sup>1</sup>, A. Kawęcka<sup>1</sup>, M. Puchala<sup>1</sup>*<sup>1</sup> National Research Institute of Animal Production, 1 Krakowska St., 32-083 Balice, Poland*

In the second decade of the 21st century, national research centers initiated efforts to recreate old, forgotten goat breeds in Poland. These efforts contributed to the enrichment of the genetic biodiversity of goat breeding in the country. Over the past 11 years, efforts have been underway to recreate the Sandomierz and Kazimierzowska goat breeds, considered extinct. These efforts were conducted in accordance with the principles established for the revival of Carpathian goats. Sandomierz goats were native to the present-day Lublin and Świętokrzyskie Voivodeships, near the confluence of the San and Vistula Rivers. An attempt to reestablish the Sandomierz goat was undertaken in 2015 by the University of Life Sciences in Lublin. Initially, a herd of 20 goats and 5 male goats was established. In 2022, when the goat genetic resources conservation program was launched, the farm had 126 goats and 13 male goats, kept in 6 herds. In 2025, the farm reached 344 goats and 46 male goats, kept in 22 herds. The Kazimierzowska goat originates from the areas around Kazimierz Dolny and Puławy, currently located in the Lublin Voivodeship. A recognizable type of this native goat emerged in the 1920s. It is characterized by a black coat, without any distinct varieties. It has long, dense fur with a noticeable undercoat. Its distinctive eyes have distinct golden-yellow irises. Work on breed restoration is underway under the auspices of the Warsaw University of Life Sciences. The first specimens were found in 2014. In 2022, 44 female goats and 10 male goats, kept in 10 herds, participated in the conservation program. Currently, goats of this breed are kept in 19 herds, with a population of 232 female goats and 31 male goats. The Sandomierz and Kazimierzowska goat breeds are being reintroduced to breeding in the Lublin and Masovian regions. These areas have also proven to be ideal locations for extensive production, and these goats can be an excellent complement to primary animal husbandry.

### Development dynamics of carpathian goats covered by the restoration program

J. Sikora<sup>1</sup>, A. Kawęcka<sup>1</sup>, M. Puchała<sup>1</sup>, I. Peist<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, 1 Krakowska St., 32-083 Balice, Poland

Attempts to revive goat breed, considered extinct, began at NRIAP with work on the reintroduction of Carpathian goat breed. In 2005, the first specimens of Carpathian type were found. Over the course of the 20 years of the 21st century, the breed was registered, a herd book was opened, a genetic resources conservation program was initiated, and the population was expanded. The aim was to assess the restored population and development of Carpathian goat breed from 2005 to 2025. Data obtained and compiled annually by NRIAP were used. Through systematic evaluation of adult goats, licensed young goats, yearling goats, and male goats, described according to the breeding standard, the number of current breeding herds was increased. Assessed herds are established and bred in the southern part of the country. The breed demonstrates good adaptation to mountainous, extensive production systems. In 2005, when breed was reintroduced, number of animals meeting the breed standard was 6 adult goats, 4 female goats, and 2 male goats. Between 2005 and 2015, number of purebred goats increased to 30 female goats and 8 male goats, located on 3 breeding farms. Between 2015 and 2020, due to expansion of breeding operations and the commencement of the purchase of breeding animals for individual herds, as well as the implementation of Carpathian goat genetic resources conservation program, there was a significant increase in breeding animals. By the end of this period, breeding of this breed was being carried out in 20 herds, containing 246 purebred goats and 32 male goats. In the subsequent period, 2020–2025, reintroduced breed saw further dynamic growth. In 2025, breeding is being carried out in 42 herds. 769 mother goats and 85 breeding goats are kept. This represents a 100-fold increase in the population over 20 years. Over past 20 years, an attempt has been made to recreate Carpathian goat breed, considered extinct. This effort involved the reintroduction of a unique domestic goat breed characteristic of the mountains. It has proven to be an excellent breeding ground, where natural conditions favor extensive production, and Carpathian goats can complement basic animal husbandry, for example, in organic farming, agritourism settings.

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### Characterization of carcass traits and meat quality of the Italian Leccese chicken

M. Santoro<sup>1</sup>, V. Landi<sup>1</sup>, L. Forte<sup>1</sup>, G. Rusco<sup>1</sup>, P. De Palo<sup>1</sup>, A. Maggolino<sup>1</sup>, N. Parabita<sup>1</sup>, G. Centoducati<sup>1</sup>

<sup>1</sup> University of Bari, Department of Veterinary Medicine, S.P. per Casamassima Km 3, 70010 Valenzano (BA), Italy

The Italian Leccese chicken (LC) is a dual-purpose breed originating from the south of Italy, traditionally reared under extensive farming systems, that plays an important role for the preservation of animal genetic diversity. Actually, data on morpho-productive traits and meat quality are still limited. The aim of this study was to provide an integrated characterization of the LC. Twenty-two LC were involved in the trial. The following parameters were recorded: live weight (LW) at 120 days, carcass weight (CW) and measurements, commercial cuts incidence, dressing percentage (DP) and pH at 1h and 24h post slaughtering. For each commercial cut, muscle and bone incidence were calculated. Moreover, chemical composition and colorimetric parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , Chroma, Hue) were measured. A descriptive statistical analysis of data was performed using Microsoft Excel® software, and the mean and SD were calculated. LW was  $2.0 \pm 0.49$  Kg, with a CW of  $1.4 \text{ Kg} \pm 0.36$  and a DP of  $70.75 \pm 2.71\%$ . The pH 24h was  $5.85 \pm 0.09$ . Chest circumference was  $29.6 \pm 2.88$  cm, limb length  $19.80 \pm 2.16$  cm and limb circumference  $12 \pm 1.87$  cm. Lightness for muscle and skin was respectively  $42.77 \pm 1.8$  and  $60.89 \pm 5.31$ . The incidence of thighs muscle was  $64.15 \pm 2.81\%$ , and of breast muscle of  $65.39 \pm 8.29\%$ . The results about carcass and muscle incidence traits are consistent with those observed in slow-growing rustic breeds. The low  $L^*$  values of the breast muscle indicate a darker colour than commonly reported for commercial lines, while the high brightness of the skin highlights how skin colour can be influenced by other factors (Devatkal S. K. et al., 2019). This study contributes to the characterisation of the LC, providing useful information for its conservation and sustainable use in local production systems. Devatkal, S. K., Naveena, B. M., & Kotaiah, T. (2019). Quality, composition, and consumer evaluation of meat from slow-growing broilers relative to commercial broilers. Poultry science, 98(11), 6177-6186.

**Discovering an Italian local breed: growth performance and meat quality of Fasanese lamb**

N. Parabita<sup>1</sup>, V. Landi<sup>1</sup>, L. Forte<sup>1</sup>, C. Pierini<sup>1</sup>, P. De Palo<sup>1</sup>, M. Santoro<sup>1</sup>, G. Calzaretti<sup>1</sup>, G. Incampo<sup>1</sup>, A. Maggiolino<sup>1</sup>

<sup>1</sup> University of Bari, Department of Veterinary Medicine, S.P. per Casamassima Km 3, 70010 Valenzano (Ba), Italy

Local breeds, as unique genetic resources, are recognized as an important element for maintaining diverse and specific agricultural systems. Furthermore, promotion of traditional products linked to local breeds is essential. The aim of this study was to characterize meat of Fasanese lambs from a chemical point of view and to evaluate the animal's morphological measurements both in vivo and post-slaughter. The trial involved 20 Fasanese lambs. From birth to slaughter live weight and morphological measurements were recorded every 15 days until slaughter at 60 days. The average daily gain (ADG) and dressing percentage (DP) were determined. pH was measured at 1h and 24h after slaughtering. Meat chemical composition was determined according to ISO1442, (1997) (1). A descriptive statistical analysis was performed using Microsoft Excel® software, and the mean values  $\pm$  SD were calculated. Slaughter weight was  $15.3 \pm 1.51$  kg, ADG was  $0.18 \pm 0.02$  kg/day, while the mean DP was  $57.84 \pm 1.47\%$ . As regards meat quality traits, pH value was  $5.81 \pm 0.20$ , moisture was  $76.58 \pm 0.87$  g/100g, protein  $18.48 \pm 0.39$  g/100g, intramuscular fat  $21.30 \pm 0.77$  g/100g and ash content  $1.15 \pm 0.77$  g/100g. The results obtained were very similar to those reported in lambs by other researchers. The DP for the Fasanese breed was higher compared to light lambs of other Italian breeds, while meat chemical composition was consistent with those reported in the literature (2). Local ovine genetic resources have developed specific adaptations to survive and produce under conditions strictly linked to their territory. These results highlight the productive suitability of the Fasanese breed for niche lamb production systems and support its conservation through product valorisation. (1) ISO1442 (1997). (Vol. ISO 1442). (2) Budimir, K., Trombeta, M. F., Francioni, M., Toderi, M., & D'Ottavio, P. (2018). Slaughter performance and carcass and meat quality of Bergamasca light lambs according to slaughter age. *Small Ruminant Research*, 164, 1-7.

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**Assessment of the effects of the sheep genetic resources conservation program in Poland**

A. Kawęcka<sup>1</sup>, M. Puchała<sup>1</sup>, M. Pasternak-Chorosz<sup>1</sup>, J. Sikora<sup>1</sup>, I. Peist<sup>1</sup>, A. Miksza-Cybulska<sup>1</sup>, R. Garstka<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, 1Department of Sheep and Goat Breeding, Krakowska 1 Str., 32-083 Balice, Poland

In many regions of the world, sheep perform not only important functions connected with product outputs, but also fulfill numerous natural and landscape functions and serve as a testament to the living traditions and culture of local communities. The aim of this study was to analyze the impact of the 20-year-long implementation of the Genetic Resources Conservation Program for Native Sheep Breeds in Poland, which began in 2005, following Poland's accession to the EU, and to assess the achievement of the program's objectives. This study analyzed data on the population of protected sheep breeds sourced from the "BIO-OWCE" database of the National Research Institute of Animal Production, covering the last 20 years (2005–2024). The study also assessed breed threat status. Since program inception, a steady increase in the protected population has been observed. Over the 20 years of its implementation, a nine-fold increase in the number of ewes from native breeds has been recorded, now constituting 80% of the breeding females in the country. Analyzing the effects of the program, it can be seen that most of the set goals have been achieved. The most measurable effect is the increase in the size of the protected population. The program began in 2005 with 11 protected breeds kept in 145 flocks and a population of 8,000 ewes. Currently, the population amounts to 75,500 ewes belonging to 17 native breeds, kept in 885 flocks. Analyzing the threat status of native breeds, it was determined that 9 are endangered, the remaining require monitoring, and none are critically endangered. Another positive aspect of implementing the genetic resources conservation program is the accompanying activities, which are based on utilizing the roles of sheep beyond productivity and also developing the market for products derived from native breeds. The implementation of conservation programs and the possibility of receiving financial support have had a significant impact on sheep breeding and the breed structure in Poland. The sheep genetic resources conservation program has proven to be an effective tool for protecting the biodiversity of this species.

### Statistical and Demographic Characterization of Breeders Involved in Conservation Programmes for Cattle and Coldblooded Horse Breeds

E. Sosin<sup>1</sup>, A. Chelmińska<sup>1</sup>, J. Wójtowicz<sup>1</sup>, M. Dziad<sup>1</sup>, G. Polak<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, Department of Farm Animal Biodiversity Conservation and Horse Breeding, Krakowska 1, 32-083 Balice, Poland

One of the challenges that animal genetic resources conservation programmes (AnGRCP) face is the aging of breeders. The study assessed the demographic and structural characteristics of 773 breeders participating in the AnGRCP in 2024-2025. Three populations of endangered breeds were analysed, including two types of cold-blooded horses (Sztumski – 266 breeders and Sokólski – 232 breeders) and one cattle breed (Polish Red-and-White – 275 breeders). The aim of the study was to assess the demographic structure of breeders involved in AnGRCP. The characteristics of horse and cattle breeders indicated common structural features of conservation breeding across species. Based on the analysed data, it was found that the typical owners of Sokólski and Sztumski horses are predominantly men (87%), most of whom are between 51 and 65 years of age (40%). The horses are kept in small herds, most often consisting of 2–3 mares (mean 5), while the largest herd included 40 mares. Similarly, breeders of Polish Red-and-White cattle were also predominantly men (84%), mainly between 51 and 65 years of age (44%). The cattle are kept in small herds comprising 9–10 cows (mean 11), and the largest herd consisted of 40 cows. Breeders of both species emphasised the importance of native breeds, and breeding activities were maintained largely due to long-standing family traditions. Almost all respondents reported that their animals had access to outdoor movement and pasture. Most farms also keep additional livestock species. The age structure of breeders indicated an ageing population. Only 18% of horse breeders and 17% of cattle breeders were young, i.e. under the age of 40. This may result in a reduced number of active breeders, difficulties in generational succession, and a potential decline in population size in the future. Overall, the results indicate that conservation breeding of native horse and cattle breeds in Poland is currently sustained by an ageing group of small-scale male breeders, which highlights the need for targeted support measures and incentives aimed at encouraging the involvement of younger generations.

### Session 2

### Theatre 1

#### A Multi-Stakeholder Approach Using Human-Centric Design for PLF Implementation in Mediterranean Upland Pastures

M. Odintsov Vaintrub<sup>1</sup>, S. S. Colasante<sup>1</sup>, M. Bianchini<sup>1</sup>, M. Francioni<sup>1</sup>, P. D'ottavio<sup>1</sup>

<sup>1</sup> Università Politecnica delle Marche, Agricoltura, Via Umbria, 63100 Ascoli Piceno, Italy

Precision Livestock Farming (PLF) has emerged as a set of technologies designed to enhance the performance, health, and welfare of animals in intensive farming systems. By leveraging sensors and data, PLF has improved operational efficiency, decision-making, and farmer quality of life. However, PLF adoption in extensive and upland livestock systems remains minimal. These farms struggle with issues like poor connectivity, low capital investment capacity, and higher aversion to innovation risk. Part of the solution lies in tech development of affordable and suitable systems. However, a more transformative approach is needed to reframe PLF system design through a stakeholder-driven lens. In this study, 12 upland pasture areas across the Central Apennines regions of Italy were analyzed. Each was evaluated in terms of production systems, land use, farm structure, and ecosystem services. Semi-structured interviews were conducted with farmers, shepherds, veterinarians and local authorities. Using human-centric design principles, individual “buyer personas” were developed to reflect the specific views of each stakeholder group. Key findings identified farmers as the primary stakeholders, with priority concerns centered on risk mitigation (e.g., animal loss, weather events), logistical planning, and access to market. Secondary stakeholders included institutional figures and technical service providers aiming to reduce their own operational costs while supporting farmers. Farmers showed openness to low-cost, generalized IoT and open data systems, while institutional stakeholders expressed interest in more complex PLF tools such as GPS collars for targeted monitoring. Therefore, a bi-polar model was developed to offer a pathway for shared responsibility in pasture data generation. In conclusion, the digitalization of Mediterranean upland pastures cannot rely on a linear technology transfer. Rather, it requires co-developing with its users, ensuring that technological functionality are aligned with local dynamics. By integrating affordable technologies with systemic Human Centric Design, PLF can become both accessible and impactful even in marginal, low-input farming contexts.

**Computer vision-based monitoring of pig behaviour in Mediterranean farms during hot periods**N. Hlel<sup>1</sup>, D. Alexander Mendez<sup>1</sup>, X. Díaz De Otálora<sup>1</sup>, F. Estellés<sup>1</sup>, S. Calvet<sup>1</sup>, D. Liu<sup>2</sup>, T. Norton<sup>2</sup><sup>1</sup> Institute of Animal Science and Technology (ICTA), Universitat Politècnica de València, Camí de Vera, 46022 Valencia, Spain, <sup>2</sup> Department of Biosystems, Division M3-BIORES: Measure, Model & Manage Bioresponses, Catholic University of Leuven, Kasteelpark Arenberg 30, Heverlee, 3001 Leuven, Belgium

Heat Stress is a major challenge in Mediterranean pig production, and animal behaviour is a crucial indicator of thermal discomfort. The use of precision Livestock Farming (PLF) offers new opportunities for continuous monitoring and early decision-making. This work evaluated the effects of warm temperatures and heat mitigation strategies on pig behaviour, using behavioural data from the M3PIG Pipeline (<https://gitlab.kuleuven.be/m3-biores/public/m3pig>). The study was conducted on an experimental farm under commercial conditions, where eight cameras were installed in top-view in eight pens, each with eight pigs. Pens differed in flooring design: four had fully slatted floors, while four had partially solid floors equipped with air-stirring fans. Animals were continuously monitored for one month. Video data were analysed at one frame per second using the M3PIG pipeline, with the main outputs being animal postures (lying, standing and sitting) and resource use (feeding and drinking), which are recognised behavioural indicators of heat stress and comfort in pigs. Behavioural indicators were derived at the group level, aggregated into 5-minute bins, and averaged daily using Python 3.12. Preliminary results showed that pigs spent most of their time lying (83.1%), followed by standing (15.2%) and sitting (1.7%), reflecting a shift toward increased lying under heat stress. Daily feeding and drinking activities averaged 44 and 25 minutes per pig, respectively. Results also indicated that pigs showed increased use of the solid floor area, with a positive correlation with the Temperature Humidity Index ( $r = 0.32$ ), indicating a preference for cooler resting areas that facilitate heat dissipation and help maintain thermal comfort and welfare. These findings demonstrate that camera-based PLF enables robust, continuous monitoring of pig behaviour under summer conditions, providing valuable information to support management decisions aimed at improving animal welfare.

## Session 2

## Theatre 3

**Digital approach for sheep reproductive management: electronic monitoring of sexual activity to improve the male effect as an alternative to hormonal treatments**H. Chalouati<sup>1</sup>, S. Khnissi<sup>1</sup>, S. Fabre<sup>2</sup>, N. Debus<sup>3</sup><sup>1</sup> INRAT, Laboratory of Animal and Forage Production, Rue Hédi Karray, Ariana, 2049 Tunis, Tunisia, <sup>2</sup> INRAE, GenPhySE, Chemin de Borde-Rouge, 31326 Castanet-Tolosan, France, <sup>3</sup> INRAE, SELMET, 2 Place Viala, 34000 Montpellier, France

This study evaluated an automated approach with low input for out-of-season reproductive management in Sicilo-Sarda ewes, a Tunisian dairy sheep breed. To assess for estrus synchronization, we compared the use of the male effect (ME) with electronic monitoring of sexual behavior using the Ovimate heat detector (Group ME, n=59) to the conventional approach using equine chorionic gonadotropin (eCG) (Group eCG, n=56), during the anestrus season. All ewes received intravaginal FGA sponges for 14 days. After sponge removal, ME ewes were exposed to intact rams fitted with aprons for six days, with estrus monitored in real time using electronic detectors, while eCG ewes received an intramuscular injection of eCG. Hand service was performed on Day 2, followed by natural mating for 17 days from Day 9. In the ME group, 95% of the ewes exhibited estrus between D0 and D6. Estrus onset occurred  $43.6 \pm 2.1$ h (mean  $\pm$  SEM) after sponge removal, with a mean duration of  $13.1 \pm 1.2$ h. Based on previous evidence indicating optimal fertility when insemination is performed within 0–35h after estrus onset, 80% of the ewes in the ME group displayed estrus within a time window compatible with fixed-time AI performed 57 h after sponge removal. The proportion of ewes mated during hand service (74.6% vs 96.4%;  $p < 0.05$ ) and the fertility in rates following hand service (50% vs 76.6%;  $p < 0.05$ ) were significantly lower in the ME group than in the eCG group. However, total fertility (89.6% vs. 93.6% for ME and eCG, respectively) and prolificacy of ewes ( $1.19 \pm 0.06$  vs.  $1.23 \pm 0.06$  for ME and eCG, respectively) did not differ significantly between groups. In conclusion, the combination of the male effect and automated monitoring of sexual behavior achieves adequate estrus synchronization for a single fixed-time artificial insemination, while reducing the reliance on hormonal treatments. This method provides an agroecological and innovative alternative to eCG for estrus induction and synchronization in out-of-season Sicilo-Sarda ewes.

### **European Network on Livestock Phenomics (EU-LI-PHE): boosting high-throughput phenotyping for precision livestock farming and animal breeding**

M. L. Calabri<sup>1</sup>, L. Fontanesi<sup>1</sup>, T. Norton<sup>2</sup>

<sup>1</sup> University of Bologna, Department of Agricultural and Food Sciences, Viale G. Fanin 46, 40127 Bologna, Italy, <sup>2</sup> KU Leuven, Department of Biosystems, Kasteelpark Arenberg 30, 3000 Leuven, Belgium

Phenomics applied to livestock production systems aims to systematically characterize the animal phenome, which includes both physical and molecular traits. The acquisition of relevant animal phenotypes is fundamental for routine management of livestock populations, enabling optimization of reproduction strategies, disease control, and animal welfare. For this reason, phenomics in animal breeding and husbandry is recognized as a key innovation in supporting the sustainability of livestock production systems. The European Network on Livestock Phenomics (EU-LI-PHE) is a Europe-centred, multidisciplinary, interconnected, and inclusive network of experts, established to strengthen scientific collaboration, catalyse developments, and promote the application of livestock phenomics to improve sustainability and competitiveness in livestock production systems. EU-LI-PHE focuses on: i) phenotyping technologies and infrastructures for livestock phenomics applications; ii) development of novel strategies for genome to phenome integration in livestock species; iii) computational resources and analytical tools for managing and interpreting large-scale datasets within livestock phenomics iv) the regulatory framework and ethical and societal aspects of livestock phenomics v) the establishment of a training environment and opportunities in order to support the next generation of researchers in livestock phenomics. EU-LI-PHE is funded by COST (European Cooperation in Science and Technology) and it brings together approximately 500 working group members from more than 50 countries. By fostering multi-disciplinary collaboration and innovation, EU-LI-PHE aims to become a reference platform for livestock phenomics in Europe and beyond, contributing to the development of sustainable, resilient, and welfare-friendly livestock production systems, while promoting novel approaches in phenotyping and Precision Livestock Farming (PLF).

### **Monitoring of dairy goat behaviour: experiences on tri-axial accelerometers and computer vision approaches**

A. Costantino<sup>1</sup>, D. A. Méndez<sup>1</sup>, B. Fajardo<sup>1</sup>, E. Gil<sup>1</sup>, S. Sanjuan<sup>2</sup>, R. Arnau<sup>2</sup>, J. M. Calabuig<sup>2</sup>, S. Calvet<sup>1</sup>, A. Villagrà<sup>3</sup>, F. Estellés<sup>1</sup>

<sup>1</sup> Universitat Politècnica de València, Institute of Animal Science and technology, Camino de Vera s/n, 46022 Valencia, Spain, <sup>2</sup> Universitat Politècnica de València, Instituto Universitario de Investigación de Matemática Pura y Aplicada, Camino de Vera s/n, 46022 Valencia, Spain, <sup>3</sup> Instituto Valenciano de Investigaciones Agrarias, Centro de tecnología Animal, Pol. La Esperanza, 100, 12400 Segorbe, Spain

Precision Livestock Farming enables continuous, objective and automated monitoring of animal behaviour. In dairy goats, behavioural monitoring is less developed than in other species. This work compares two complementary approaches for monitoring behaviour in dairy goats: tri-axial accelerometers and computer vision. The first approach uses collar-mounted accelerometers in Murciano-Granadina goats. Behavioural observations (115 h) were video-labelled to train a multilayer perceptron to classify eating, walking and inactive states. The model reached ~0.90 accuracy. This strategy allows continuous monitoring independent of lighting and pen design, linking data directly to animals. However, it requires device attachment, battery maintenance, and may be affected by collar displacement or motion artefacts. The second approach uses overhead RGB cameras and multi-object detection for contactless group monitoring. A model trained on 9,976 labelled instances achieved mean average precision up to 0.96 with real-time speeds (>50 images·s<sup>-1</sup>). It classified eating, standing, lying and drinking from top-view images without virtual zones. This system avoids instrumentation and provides spatial information on group distribution and interactions. Yet performance is influenced by occlusions, lighting variability, camera distortion and class imbalance, and individual tracking still shows ID-switching. Accelerometers provide robust, individual and lighting-independent data but are invasive and less scalable. Computer vision is non-invasive and scalable, but depends on infrastructure, data quality and algorithm robustness, and does not ensure individual identification. Together, both show strong potential for integrated behavioural phenotyping and early welfare or health detection in dairy goat systems.

**Integrated UAV and IoT Ecosystem for Livestock Monitoring: The SPADE Livestock Pilot Case Study**A. Frantzis<sup>1</sup>, M. Karatzia<sup>2</sup>, E. Gkimousi<sup>1</sup>, G. Manassis<sup>1</sup>, C. Davarakis<sup>3</sup>, I. Bossis<sup>1</sup>, Z. Basdagianni<sup>1</sup><sup>1</sup> Aristotle University of Thessaloniki, Department of Animal Production, School of Agriculture, AUTH Campus, 54124 Thessaloniki, Greece, <sup>2</sup> ELGO-DIMITRA, Research Institute of Animal Science, Paralimni, 58100 Giannitsa, Greece, <sup>3</sup> Nydor System Technologies S.E., N. Psychiko, 11525 Athens, Greece

Traditional livestock monitoring for small ruminants in complex environments remains labor-intensive, costly, and challenging for animal welfare and grazing management. This study addresses these limitations by developing a physical–cyber UAV and IoT ecosystem based on a Digital Twin Framework. Individual animals and flocks are digitally represented to support data-driven decision-making. The system was designed in alignment with farmers' operational needs to reduce and simplify routine tasks, and improve situational awareness. Field trials were conducted at multiple pilot sites in N. Greece, including research farms and commercial-like environments, using commercial and custom UAVs equipped with RGB and thermal cameras. Direct sensing used selected biologgers fitted with cellular IoT wearables integrating GNSS and 3-axis accelerometers operating at 20Hz for real-time tracking and behavioral analysis. UAV-based remote sensing and wearable IoT devices enabled integrated pasture- and animal-level monitoring, while collars provided continuous individual tracking and behavioral data, enabling robust flock monitoring. Data from both sensing layers were processed at the edge or in the cloud using AI object detection models (YOLOv8) and uploaded to the Digital Twin Manager. Trials demonstrated high-accuracy detection and counting of sheep/goats (up to 95%) and stable real-time herd tracking. The ecosystem also establishes foundations for livestock health monitoring through UAV imagery and annotated datasets. In conclusion, combining UAV remote sensing with wearable direct sensing addresses key limitations of traditional monitoring. Integration within a Digital Twin framework advances precision livestock farming and animal welfare in resource-constrained, demanding environments. This project has received funding from the European Union's Horizon Europe research and innovation programme under Grant Agreement no. 101060778.

**Monitoring grazing horses in a Mediterranean inner area by GPS collars**A. Fatica<sup>1</sup>, F. Fantuz<sup>2</sup>, D. Tedesco<sup>3</sup>, L. Todini<sup>2</sup>, G. Pastorelli<sup>4</sup>, E. Salimei<sup>1</sup><sup>1</sup> University of Molise, Agriculture, Environment, and Food Sciences, via F. de Sanctis, 86100 Campobasso, Italy, <sup>2</sup> University of Camerino, School of Biosciences and Veterinary Medicine, via Madonna delle Carceri, 62032 Camerino, Italy, <sup>3</sup> University of Milan, Environmental Science and Policy, via Celoria, 20133 Milano, Italy, <sup>4</sup> University of Milan, Veterinary and Animal Science, via dell'Università, 26900 Lodi, Italy

The present study deals with GPS collar data related to season and time dependent locomotion and body temperature of grazing horses in the Apennine pasture of Capracotta (41°50'N, 14°16'E; Isernia province, Italy), located between 850 and 1500 m a.s.l. Twelve grazing Italian Heavy Draught Horses were monitored from August 2024 to November 2025 by GPS collars (Digitanimal®, Spain), recording geographic position and external body temperature every 30-minutes. Locomotion data, including distance, average speed and activity frequency, were calculated from consecutive GPS fixes. Data were processed by horse, season and day intervals (morning, afternoon, night). The effects of season, time and their interaction were assessed by linear mixed models using SPSS (Chicago USA, v. 25) and R (Core Team, v. 4.5.2). Spatial processing and visualization were performed by QGIS (D. Team, v. 3.34.6). Average daily distance (5.1-5.3 km) and speed (170-176 m/min) showed significant seasonal ( $p < 0.05$ ) and time ( $p < 0.001$ ) differences, with lowest values recorded in summer and afternoon. The average activity frequency differed among seasons ( $p < 0.001$ ), with higher values in summer (8.5 km/day) and autumn (7.1 km/day). The observed lower nocturnal activity frequency highlights scattered but faster movement events. Mean external body temperature showed seasonal and diurnal variations ( $p < 0.001$ ), indicating adaptive thermoregulation under extensive grazing conditions supporting animal welfare status. In mountain pastures, GPS tool promotes sustainable grazing management by preventing unbalanced grazing activity, contributing to land use preservation in Mediterranean inner areas. The study, approved by University of Molise Bioethics Committee, was supported by project PRIN 20224L4WSR, funded by Next Generation EU (CUP: H53D23005120).

### Meta-analysis of validation evidence for wearable sensors compared with visual observation of feeding behaviors in dairy cattle

B. Darabighane<sup>1</sup>, A. S. Atzori<sup>1</sup>

<sup>1</sup> University of Sassari, Department of Agricultural Sciences, Sassari, 07100 Sassari, Italy

One of the key components of health monitoring and management in dairy cows is the continuous, non-invasive measurement of feeding behaviours, particularly eating time and rumination time. Wearable sensors (ear tags, halters, and neck collars) can capture and transmit real-time data, but their agreement with human visual observation varies by device and setting. A meta-analysis was conducted to quantify both Pearson's correlation coefficient (PCC) and Lin's concordance correlation coefficient (CCC) between wearable sensors and visual observation of eating and rumination times in dairy cattle in non-grazing systems. Bibliographic searches from 2000 to 2025 were performed in Web of Science, Scopus, and Google Scholar. For each outcome, effect sizes were transformed to Fisher's  $z$ , pooled using an inverse-variance random-effects model, and the pooled estimate with its 95% CI was back-transformed to the original scale. Heterogeneity was assessed using  $Q$  and  $I^2$ . Subgroup analyses compared wearable sensors, and publication bias was evaluated with Egger's test. The overall effect size for PCC for eating time and rumination time was 0.911 and 0.901, respectively, with significant heterogeneity for both outcomes ( $P \leq 0.001$ ). For both outcomes, subgroup analyses showed no statistically significant differences among wearable sensor types; however, the largest PCC was observed for eating time in the neck collar group and for rumination time in the halter group. The overall effect size for CCC for eating time and rumination time was 0.872 and 0.889, respectively, with significant heterogeneity ( $P < 0.001$ ). Egger's tests for PCC and CCC across both outcomes showed no evidence of publication bias ( $P > 0.1$ ). Overall, wearable sensors were highly correlated with visual observation for quantifying eating time and rumination time, although agreement was inconsistent across studies. Because of location and scale biases reflected in a lower bias correction factor, CCC was generally lower than PCC. No significant differences emerged among sensor types, yet performance varied with the specific sensor as well as housing conditions and physiological status.

### Session 2

### Theatre 10

#### Video annotation system for lambing detection.

M. Garcia-Herreros<sup>1</sup>, M. R. Marques<sup>1</sup>, A. T. Belo<sup>1</sup>, M. Antunes<sup>2</sup>, P. Gonçalves<sup>3</sup>

<sup>1</sup> Instituto Nacional de Investigação Agrária e Veterinária, I.P., Av. Professor Vaz Portugal, 2000-424 Vale

de Santarém, Portugal, <sup>2</sup> DETI and IT, Universidade de Aveiro, 3830-193 Aveiro, Portugal, <sup>3</sup> ESTGA and IT, Universidade de Aveiro, 3830-193 Aveiro, Portugal

Automatic lambing detection represents a transformative tool for improving sheep flocks' efficiency and welfare by providing real-time alerts that free staff from continuous manual monitoring. Video-based monitoring allows a single camera to monitor multiple individuals simultaneously, providing spatial coverage. This research details a monitoring trial of 26 lambing ewes, proposing a framework designed to manage large-format video data through a tiered pre-processing pipeline. To address the computational burden of 24-hour (plus) high-resolution streams, where only a fraction of frames contains relevant animal behaviour, the proposed system leverages a temporal sampling strategy combined with Motion Vector Analysis. This initial stage filters static intervals and redundant background noise. The filtered stream is then processed by a Temporal Shift Module integrated with a CNN backbone. By shifting part of the feature channels along the temporal dimension, the model captures motion dynamics across multiple frames per second without the heavy overhead of optical flow or 3D convolutions, maintaining the low latency required for real-time edge deployment. Finally, the system utilises a pre-trained feature extractor tuned on general animal behaviour datasets providing operational sensitivity, and its results are validated against human monitoring annotations. As this system remains in the prototype phase, the current efforts are focused on refining the transition between the automated inference and the human-in-the-loop validation triggers. To ensure the model's robustness, the next stage involves stress-testing the incremental learning loop across varying lighting conditions and barn densities. While the preliminary results were promising, the project remains open to further optimising multimodal fusion weights to better handle the asynchronous nature of video and sensor data. Finally, this prototype is an important first step towards fully automatic detection of the parturition process that balances quick processing with the high accuracy needed for veterinary care.

### **First application of virtual fencing in an intensive rotational grazing system in Central Italy: cattle responses during training and early transitions**

G. Bernabucci<sup>1</sup>, L. Basiricò<sup>1</sup>, B. Ronchi<sup>1</sup>, N. Lacetera<sup>1</sup>, R. Primi<sup>1</sup>

<sup>1</sup> University of Tuscia, DAFNE, Via san camillo de lellis snc, 01100 Viterbo, Italy

Intensive rotational grazing (IRG) systems improve pasture use, but requires frequent animal movements and fence management. Virtual fencing (VF), based on GPS collars delivering acoustic warnings (A) followed, if necessary, by mild electrical stimuli (I), may reduce these constraints. Although previous studies suggest that VF is effective in large open grazing areas, evidence of its reliability in small paddocks, typical of IRG, is still lacking. Within the AGRITECH project (NextGeneration EU), this ongoing study evaluates VF integration into an IRG system, focusing on animal responses during training and first paddocks transitions. The trial was carried out on a commercial beef farm in Central Italy within a Voisin-managed multi-paddock system (0.3–0.6ha). The herd consisted of 24 adult Black Angus (23 cows and 1 bull) and 12 calves, with an instantaneous stocking density ranging from 43 to 90 LU/ha. The animals grazed each paddock for three days and received hay supplementation on two of those days. Cows were fitted with VF collars (eShepherd, Gallagher, Victoria, Australia) in late November 2025 and trained over 6 days in 3 phases: (P1) VF overlapping the electric physical fence (1 day); (P2) virtual boundary restriction of 3m inside the physical fence (4 days); (P3) paddock subdivision using VF only (1 day). Animals then started IRG (R1). Data on audio warnings and electrical impulses per animal were analysed using a Generalised Linear Model, with animals (23 levels) and period (4 levels) as fixed effects. Differences among phases were assessed at  $p < 0.05$ . During P1, animals received few stimuli, with mean $\pm$ SD of 5.61 $\pm$ 0.92 A and 2.22 $\pm$ 0.32 I per animal. In P2, interactions with VF increased significantly ( $p < 0.01$ ), with 12.22 $\pm$ 0.85 A and 1.55 $\pm$ 0.14 I per animal. On P3 the highest number of stimuli was reached, with 15.26 $\pm$ 1.81 A and 3.96 $\pm$ 0.50 I per animal. During R1, A remained stable (14.39 $\pm$ 1.77), whereas I markedly decreased (0.59 $\pm$ 0.12;  $p < 0.01$ ). These preliminary findings indicate rapid learning and habituation within 6 days of training, demonstrating that cattle can quickly adapt to VF and supporting its feasibility in an IRG system.

### Session 2

### Theatre 12

#### **Application of Augmented reality technologies for precision livestock management**

G. Sara<sup>1</sup>, D. Pinna<sup>1</sup>, G. Todde<sup>1</sup>, M. Caria<sup>1</sup>

<sup>1</sup> University of Sassari, Department of Agricultural Sciences, Viale Italia 39/a, 07100 Sassari, Italy

Precision livestock farming needs tools that provide real-time, animal-specific information in the field to improve efficiency, welfare, and sustainability. Over the last decade, Augmented Reality (AR) have attracted strong interest and are potentially valuable in agriculture. Adoption in livestock farming is limited due to the lack of tailored applications and poor interoperability with existing farm management and sensing systems. This work provides the research activities roadmap, which brings to the development of a decision support system that integrates electronic animal identification and an AR interface for data visualization. The workflow comprised: (i) a technical benchmarking of AR devices; (ii) stakeholder engagement through structured surveys and practical tests; (iii) prototyping and validation of animal identification devices to a custom AR application. The achieved results indicate a progressive advancement from feasibility assessment to integrated, field-tested prototypes. Comparative evaluations of different AR devices clarified the performance constraints and usability requirements associated with livestock environments, enabling the selection of a device suitable for custom development and system integration. Stakeholder-focused analyses provided evidence on the main determinants of technology uptake, reinforcing that perceived usefulness, ease of use, and compatibility with existing farm systems are critical for effective deployment. Furthermore, to enable animal-specific AR, a wearable electronic identification reader was developed to link animal tags with AR device. This framework was successfully validated under both laboratory and farm conditions. Future steps of the research activities will focus on the integration of real-time location system (e.g., Ultra-wide band technology) with the AR interface, enabling spatial visualization of animal locations together with their specific information.

### **Calibration of the ModVege grassland model for Mediterranean conditions: application to the Abruzzo region (Central Italy)**

A. Canon<sup>1</sup>, T. Norton<sup>1</sup>, M. Odintsov Vaintrub<sup>2</sup>

<sup>1</sup> KULeuven, Department of Biosystems, Kasteelpark Arenberg 30, 3001 Leuven, Belgium, <sup>2</sup> REGROWTH SRLS, Contrada Specola SNC, 64100 Teramo, Italy

Mediterranean permanent grasslands are characterised by strong seasonal contrasts in herbage growth and structure, driven by temperature, water availability, and plant community composition. To explore these dynamics, we used ModVege, a mechanistic model that represents the dynamics of production, structure and digestibility in permanent pastures through a set of climate- and plant community-driven growth and senescence processes. Originally developed by Jouven et al. (2006) for managed temperate pastures, ModVege provides a parsimonious framework that can be adapted to diverse environmental contexts. The aim of this study was to calibrate the ModVege model for the Abruzzo region (Central Italy), while maintaining the model's original structure and interpretation. Calibration targeted both climatic parameters controlling potential growth and limitation functions and plant community parameters governing maximum biomass, growth allocation, tissue turnover, and senescence rates. Daily climate inputs were obtained from the ERA5-Land dataset (Muñoz Sabater, 2019), and parameter values were chosen within ranges reported for Mediterranean grasslands. A stepwise calibration approach was applied, combining qualitative sensitivity analysis with iterative parameter adjustment to ensure internal consistency in simulated green and dead biomass dynamics throughout the seasons. Initial simulations show seasonal biomass dynamics consistent with expected Mediterranean grassland functioning, with limited winter decline, rapid spring growth, and pronounced summer senescence. While these outcomes are encouraging, formal validation against field measurements is still required. Beyond calibration, the ModVege model offers a solid biophysical basis for integration with Precision Livestock Farming (PLF) approaches, supporting improved interpretation of animal–pasture interactions and data-driven grazing management under Mediterranean conditions. Overall, this study delivers a transparent and reproducible calibration framework and represents a first step toward scenario-based applications in the Abruzzo region.

### Session 2

### Poster 14

### **LIVECoVET: Advancing skills for sustainable and innovative livestock farming in Europe**

A. Canon<sup>1</sup>, T. Norton<sup>1</sup>

<sup>1</sup> KULeuven, M3-BIORES-Measure, Model & Manage Bioresponse, Department of Biosystems, Kasteelpark Arenberg 30, 3001 Leuven, Belgium

The LIVECoVET project addresses critical skills gaps in livestock farming across eight European countries (Belgium, Greece, Portugal, Romania, France, Luxembourg, Spain and Italy) by examining how vocational education and training (VET) supports the transition toward more sustainable, agroecological and digitally enabled livestock systems. The project is funded under the Erasmus+ Programme 2024 call of Partnership for Excellence: Centres of Vocational Excellence (CoVEs). The analysis is based on a convergent mixed-methods approach combining electronic questionnaires, semi-structured interviews and focus groups. More than 180 stakeholders, including farmers, advisors and VET providers, were consulted using a common framework across countries and livestock sectors. Quantitative data were analysed using descriptive statistics, while qualitative inputs were synthesised through structured summaries and word-frequency analysis. Findings from different sources and countries were compared and triangulated to identify recurring patterns, gaps and opportunities. Results show that while many sustainable practices and digital technologies are already available, their adoption is frequently limited by skills gaps, low confidence in digital tools and weak alignment between training provision and on-farm realities. In contrast, contexts with strong advisory support, peer learning and collaboration networks tend to show higher uptake of innovative practices. The study highlights the importance of integrating agroecological and digital approaches within coherent learning pathways. Agroecology supports a systemic understanding of pasture management, nutrient cycles, animal health prevention and ecosystem functioning, while digital tools enhance observation, monitoring and decision-making, particularly in extensive and remote systems. In this context, Precision Livestock Farming (PLF) is identified as a key digital approach requiring targeted skills development. Overall, the results underline the need for modular, practice-oriented and farm-based training programmes that combine technical and transversal skills with experiential and peer learning, strengthening VET as a lever for the sustainable transition of European livestock farming.

### **SENSTARA: Advancing sensor-based indicators of animal resilience, health, and welfare in precision livestock farming**

D. Foyl<sup>1</sup>, J. M. Gautier<sup>2</sup>, B. Foris<sup>3</sup>, C. Morgan-Davis<sup>4</sup>, L. Dale<sup>5</sup>, P. P. Nielsen<sup>6</sup>, G. A. Franchi<sup>7</sup>, X. Díaz De Otálora<sup>8</sup>

<sup>1</sup> AgriGates LLC, Philadelphia, United States, <sup>2</sup> Institut de l'Élevage, CS 52637, 31321 Castanet Tolosan, France, <sup>3</sup> University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria, <sup>4</sup> Scotland's Rural College, Kirkton, FK20 8RU Criannlarich, United Kingdom, <sup>5</sup> LKV Baden-Württemberg, Heinrich-Baumann-Str. 1-3, 70190 Stuttgart, Germany, <sup>6</sup> RISE Research Institutes of Sweden, Scheelevägen 17, 223 70 Lund, Sweden, <sup>7</sup> Aarhus University, Blichers Allé 20, 8830 Tjele, Denmark, <sup>8</sup> Universitat Politècnica de València (UPV), Camino de Vera s/n, 46022 Valencia, Spain

Mediterranean livestock systems are increasingly challenged by climate change, production intensification, and growing societal expectations regarding animal welfare, environmental sustainability, and transparency. In this context, Precision Livestock Farming (PLF) technologies offer powerful tools for continuous animal monitoring. However, translating large and heterogeneous sensor datasets into robust, biologically meaningful indicators that can be consistently applied across species, production systems, and environmental conditions remains a major challenge. SENSTARA (Sensor and Standards Development for Research Activities) is an international Working Group within the EAAP-PLF Commission that aims to advance sensor-based assessment of animal resilience, health, and welfare. Through advanced sensing technologies, modelling, and artificial intelligence, SENSTARA promotes the development, validation, and harmonisation of sensor-derived indicators, metrics, and models, thereby improving methodological standardisation across species and production systems. Its scientific and technological scope encompasses wearable, environmental, and physiological sensors; imaging technologies such as infrared thermography and video-based monitoring; acoustic and environmental sensing systems; and advanced data analytics, modelling, and artificial intelligence approaches. By fostering collaboration among researchers, technology developers, industry stakeholders, and policy actors, SENSTARA provides an open and interdisciplinary platform to accelerate the translation of PLF innovations into practical, transparent, standardised, and scalable decision-support tools.

### **Integrating Precision Livestock Farming to Enhance Sustainability and Performance of Pasture-Based Beef Fattening Systems**

F. Sirtori<sup>1</sup>, A. Confessore<sup>1</sup>, R. Derudas<sup>2</sup>, A. Monagheddu<sup>2</sup>, S. Khater<sup>2</sup>, P. Arca<sup>2</sup>, A. Franca<sup>2</sup>, E. Vagnoni<sup>3</sup>, C. Aquilani<sup>1</sup>, S. Parrini<sup>1</sup>, L. De Guttry<sup>1</sup>, C. Dibari<sup>1</sup>, G. Argenti<sup>1</sup>, D. Neddud<sup>2</sup>, D. Dettori<sup>2</sup>, C. Pugliese<sup>1</sup>

<sup>1</sup> University of Florence, Department of Agriculture, Food, Environment, and Forestry (DAGRI), Piazzale delle Cascine 18, 50144 Florence, Italy, <sup>2</sup> National Research Council, Institute for the Animal Production System in the Mediterranean Environment (ISPAAM-CNR), Traversa La Crucca, 3, 07100 Sassari, Italy, <sup>3</sup> National Research Council, Institute of Bioeconomy (IBE-CNR), Traversa La Crucca, 3, 07100 Sassari, Italy

The INSTINCT project (PRIN Programme 2022) aims to improve pasture-based beef systems through use of Virtual Fencing (VF). The project evaluated VF-assisted rotational grazing of Sarda-Limousine calves in Sardinia (Italy) from weaning to slaughter. From April to June 2025, six calves (average body weight 340 kg; age 15.5 months) were equipped with VF collars to continuously monitor location and activity. Calves received 3 kg/head/day of commercial concentrate, with remaining nutritional requirements supplied by rotational grazing. Pasture monitoring included herbage dry matter production under grazed and ungrazed conditions, floristic composition, and forage nutritive value. Animal performance indicators were recorded throughout the grazing season, and meat quality traits were evaluated post-mortem. Results showed that VF effectively confined animals within designated grazing areas. A progressive decrease in the ratio of electric pulses to acoustic warnings over time indicated animal adaptation to the system. Herbage availability averaged 4,874 kg DM ha<sup>-1</sup>, with corresponding forage quality (mean, % DM) of 20.79 CP, 47.45 NDF, and 29.50 ADF. Animal performance was satisfactory, with an average daily gain of 0.9 kg/day, comparable to conventional indoor systems, and no negative effects on meat quality were observed. VF enabled dynamic, adaptive grazing management, improving remote control of grazing areas and reducing animal handling, while showing strong potential to reduce labor requirements, improve grazing efficiency, and enhance system sustainability through optimized land use. A Life Cycle Thinking analysis will assess the system's environmental and economic impact.

**Optimizing Dairy Farm Efficiency: A case study in Process Mapping and Business Process Management for feeding operations**

M. G. Podda<sup>1,2</sup>, G. S. Sechi<sup>1,2</sup>, A. S. Atzori<sup>2</sup>

<sup>1</sup> IUSS Pavia, Piazza della Vittoria 15, 27100 Pavia, Italy, <sup>2</sup> Università degli studi di Sassari, Dipartimento di Agraria, Viale Italia 39, 07100 Sassari, Italy

While advanced technologies and best practices are widely promoted in dairy farming, they typically provide isolated recommendations without addressing the systematic organization of processes, including the definition of responsibilities, timing, decision criteria and interconnections among activities. This study applies a Business Process Mapping (BPM) approach, widely used in manufacturing and logistics but still rarely adopted in the dairy sector. A BPM approach supported by KPIs was used to analyze feeding in two commercial dairy farms in Arborea, Italy: Farm A (227 cows) and Farm B (110 cows). The focus was TMR preparation and distribution, due to its central role in resource management and animal performance. The feeding system was described using flowcharts and SIPOC diagrams to reconstruct the “As-Is” scenario. The process was divided into three sub-processes: (i) warehouse and ingredient management, (ii) TMR preparation, and (iii) feed distribution and animal response. Process times were recorded, and a KPI framework evaluated performance across four functional areas. Data were collected during routine operations, with times standardized per 110 milking cows. Key phases identified were loading, mixing, distribution, and TMR quality. Technical indicators included premix and TMR homogeneity and deviations from formulated rations. Nutritional and physical non-homogeneity may reduce intake and promote feed sorting, negatively affecting performance. Preparation time was 59 min in Farm A and 26 min in Farm B, with differences in loading (13 vs. 4 min), movements (11 vs. 4 min), mixing (9 vs. 2 min), and unloading (4.3 vs. 2 min). Loading and movements were the most time-consuming phases. Animal response indicators included feed efficiency, milk yield, and metabolic health. These findings demonstrate that BPM principles can be translated to livestock operations, providing systematic analytical tools to transform isolated best practices into structured, optimized processes for sustainable farm management. This research was conducted within the Italian national inter-university PhD program in Sustainable Development and Climate Change.

**Plenary Session****Theatre 1****From sensing to understanding: artificial intelligence and the future of pig welfare assessment**

G. Pulina<sup>1</sup>, M. G. Cappai<sup>2</sup>, M. F. Lunesu<sup>1</sup>, G. Battacone<sup>1</sup>

<sup>1</sup> University of Sassari, Department of Agriculture, Viale Italia 39, 07100 Sassari, Italy, <sup>2</sup> University of Sassari, Department of Veterinary Medicine, Via Vienna 2, 07100 Sassari, Italy

Recent advances in artificial intelligence (AI) are transforming the way animal behaviour and welfare can be observed and interpreted in livestock systems. In pigs, growing evidence of cognitive and emotional complexity calls for monitoring approaches capable of capturing behavioural and physiological signals beyond traditional human observation. This contribution discusses how AI-based technologies can extend human perception and support a more objective and continuous assessment of animal welfare. Current applications in swine production already include automated video analysis, facial recognition, acoustic monitoring and multimodal behavioural tracking based on deep neural networks. These tools allow the detection of stress, health disorders and behavioural changes that would otherwise remain difficult to identify. Looking forward, the convergence between advanced neural networks, large language models (LLMs) and embodied or physical artificial intelligence systems is expected to further expand these capabilities. Embodied AI systems integrating sensors, machine learning and adaptive control may enable real-time interpretation of animal states within the production environment, opening new perspectives for precision livestock farming. By aligning sensing technologies with species-specific perceptual and behavioural characteristics, future AI systems could improve the reliability of welfare assessment and support more responsible and transparent animal management. These developments suggest that artificial intelligence may become a key tool for bridging the gap between human observation and the lived experience of farm animals.

**Artificial Intelligence in Animal Science: a perspective**D. Gianola<sup>1</sup><sup>1</sup> *University of Wisconsin–Madison, 500 Lincoln Drive, 53706 Madison, United States*

Artificial intelligence (AI) refers to computational systems that involve human intelligence. This talk deals with how quantitative animal genetics evolved from “basic statistical wisdom” to deep learning. Linear models, probability theory, neural networks and kernel methods led to statistical machine learning. AI learns structure to acquire knowledge and to develop decisions. Data is fed to some model (“machine”) in a “training” process and, given input variables, we predict or classify new targets. Predictions are compared with masked realized data in a “testing set”. Measures of accuracy enable comparison of machines. Interventions developed by AI are field-orientated. Some algorithms produce whole-system solutions by “integrating” contributions from component perspectives. Genetic algorithms emulate evolution by comparing proposals, mutate and recombine components, to then select survivors. “Fake” solutions may suggest hypotheses that the human brain may have not imagined. Under changing conditions (genetic improvement, climatic change) training data may not be representative of testing data, and AI has a non-null failure rate. In 1974 the Nobel Prize in economics (Hayek) expressed a lack of satisfaction with econometric models applied to societies because conditions change! The last generation of AI includes large language models (LLM); these have not been applied yet to genetic improvement. Sentences represent coherent and words, so LLM potentially embeds epistasis. Another development is the nucleotide transformer or genomic foundation model. It can be trained with DNA from several species and has billions of parameters. Transfer learning may allow the borrowing of information from large breeds of cattle, e.g., Holsteins, to predict performance in smaller breeds, e.g., Normande. Complex systems cannot be learned from factorial or randomized experiments. The assumptions of independence and identity in distribution, or even of randomness, hardly apply to observational data. Statistical education in the animal sciences needs to change. AI does not yield measures of uncertainty. Bayesian epistemology with its probabilistic structure represents how knowledge accrues and highlights that a point prediction is incomplete information in the absence of a measure of uncertainty.

**Beyond nutrients: microalgae and yeast as functional tools in sustainable small ruminant production**A. Mavrommatis<sup>1</sup>, C. Christodoulou<sup>1</sup>, P. Kyriakaki<sup>1</sup>, R. Andreaki<sup>1</sup>, E. Tsiplakou<sup>1</sup><sup>1</sup> *Agricultural University of Athens, School of Animal Biosciences, Department of Animal Science, Laboratory of Nutritional Physiology and Feeding, Iera Odos 75, GR-11855 Athens, Greece*

Single-cell biomass in animal nutrition was initially explored to partially replace conventional feedstuffs by leveraging the high nutrient density of algae, yeast, and other microbial products. While full-scale replacement remains economically challenging, low-level inclusion of these biomasses as functional additives has shown clear biological benefits. Research has shifted from simply maximizing biomass yield to understanding bioactive compounds, functional properties, and practical applications. Microalgae such as *Schizochytrium* sp., *Nannochloropsis gaditana*, and *Spirulina platensis*, along with probiotic yeast like *Saccharomyces cerevisiae*, have demonstrated significant effects in sheep and goat diets. These additives can modulate rumen fermentation, shape microbial communities, and improve nutrient utilization. They enhance antioxidant capacity, modify milk fatty acid profiles, and improve the nutritional and functional quality of milk and dairy products. By linking rumen microbiology, systemic metabolism, and product quality, the findings provide mechanistic insight and practical evidence supporting the use of algae- and yeast-based additives in sustainable small ruminant production. An additional dimension of this research highlights the strategic use of these additives during early life. Nutritional interventions at this critical developmental window may program rumen functionality, metabolic resilience, and long-term productive efficiency, extending benefits far beyond immediate performance.

**Pastoralism and agro-pastoralism in tension: a year (and furthermore?) to empower the strengths of these livestock farming systems, the opportunities addressed to them and minimize their weakness and the threats they face, toward sustainability**

M. O. Nozières-Petit<sup>1</sup>, B. Hubert<sup>2</sup>, B. Dedieu<sup>1</sup>

<sup>1</sup> UMR Selmet, INRAE, Cirad, Institut Agro Montpellier, 2 place Viala, 34060 Montpellier, France, <sup>2</sup> UR Ecodeveloppement, 228, route de l'Aérodrome Domaine Saint Paul Site Agroparc, CS 40509 84914 Avignon Cedex 9, Avignon, France

2026 is the International Year of Rangelands and Pastoralists (IYRP). Proclaimed by the United Nations and supported by the FAO, it aims to highlight this particular form of livestock farming, which is largely widespread throughout the world, including Europe and Mediterranean areas. Fundamentally shaped by the grazing of spontaneous vegetation by animals, pastoral or agro-pastoral systems are able to cope with hazards, based on their resilience, and to provide multifunctionality. These functions and processes are interesting to consider for the transformation of other modes of livestock farming toward sustainability. This synthesis is based on a literature review. We will present, first, the several definitions of this activity, its extent in the world and the diversity of its forms. Second, identified by a group of 40 French researchers we coordinate at the occasion of the IYRP (Dedieu et al., 2025), we will explicit and exemplify the six challenges addressed to these livestock farming systems: social and environmental changes, territorial dynamics, climate change, evolution of public policies, sustainability of food systems, and workforce needs. Growing in scale and intensity, they are now undermining pastoral and agro-pastoral activities around the world, even though those are accustomed to dealing with uncertainty and complexity. These challenges justify further researches, and the French research group proposed five key areas of research that will be presented and exemplified in the third part of this presentation. Building on these strengths and new knowledge, pastoral and agro-pastoral livestock farming systems will serve as cornerstones of sustainable development worldwide, either by reintroducing them to areas where they have disappeared or by adopting the “key operating functioning rules” that characterize them in other livestock farming systems.

### Session 3

### Theatre 1

**Simple genomic analysis of environmental adaptation**

A. Cesarani<sup>1</sup>, M. Congiu<sup>1</sup>, S. Mastrangelo<sup>2</sup>, G. Senczuk<sup>3</sup>, S. Ben Jemaa<sup>2</sup>, C. Dimauro<sup>1</sup>, N. P. P. Macciotta<sup>1</sup>

<sup>1</sup> University of Sassari, viale italia, 07100 Sassari, Italy, <sup>2</sup> University of Palermo, Viale delle Scienze, 90128 Palermo, Italy, <sup>3</sup> University of Molise, Via De Sanctis, 86100 Campobasso, Italy

Environmental adaptation (EA) is essential in livestock, enabling animals to maintain health, productivity, and resilience under diverse climatic and geographic conditions. EA is particularly relevant in the Mediterranean region, which is considered one of the areas most affected by climate change. Aim of this study was to test an easy and fast way to identify genomic regions potentially associated with environmental adaptation in Sarda sheep. We analyzed 1534 animals from 11 flocks distributed across the island of Sardinia. The flocks were chosen to prevent, or at least limit, animal exchange among climate zones. After quality control, 31671 SNPs with at least 20 minor homozygotes were considered. Historical data from the past 20 years were collected for eight climate variables: sky clarity index (SCI); specific (SH) and relative (RH) humidity; mean (MeanT), maximum (MaxT), and minimum (MinT) temperature; average (AWS) and maximum (MWS) wind speed. These variables were analyzed with SNPs fitted as cross-classified fixed effects, and p-values were adjusted using Bonferroni. A total of 352 markers showed significance across all climate variables, although none remained significant for all traits after Bonferroni correction. Three SNPs showed Bonferroni significant associations: rs426284690 (CHR3) with all traits except SCI, rs398939744 (CHR6) with SCI, and rs420752986 (CHR21) with MWS. Six genes were found: PLEKHA5 on CHR3; PPARGC1A, DHX15, SOD3, and CCDC149 on CHR6; NELL1, on CHR21. The PLEKHA5 gene has been associated with milk, fat, and protein yields and fat and protein percentages. Polymorphisms within the PPARGC1A gene were significantly associated with growth traits in sheep and growth and carcass traits in cattle. The SOD3 gene was related with fat deposition in sheep and heat tolerance in goats. Results demonstrate a rapid and effective approach for identifying genomic regions involved in environmental adaptation in dairy sheep and confirmed phenotypic differences among climate zones. This study was funded by the European Union Next-Generation EU (GEREMES PRIN project, CUP J53D23013860001; AGRITECH PNRR).

### Identification of genetic variants associated with milk production, milk composition and methane emission traits in dairy sheep

G. Conte<sup>1</sup>, E. Senatore<sup>1</sup>, G. Foggi<sup>1</sup>, A. Silvi<sup>1</sup>, L. Turini<sup>1</sup>, A. Cesarani<sup>2</sup>, A. Negro<sup>3</sup>, S. Grande<sup>3</sup>, N. P. P. Macciotta<sup>2</sup>, M. Mele<sup>1</sup>  
<sup>1</sup> University of Pisa, via del Borghetto, 80, 56124 Pisa, Italy, <sup>2</sup> University of Sassari, viale Italia, 39, 07100 Sassari, Italy, <sup>3</sup> ASSONAPA, Località Le Cortine, 53041 Asciano (SI), Italy

Ruminants derive nutrients from the microbial fermentation of plant material occurring mainly in the rumen, a complex, multilayered forestomach. A major drawback of this fermentation is the production of enteric methane (CH<sub>4</sub>), which represents both an energy loss and an environmental concern. Genetic selection is a promising strategy to mitigate CH<sub>4</sub> emissions in ruminants; however, its implementation requires methane phenotypes to be collected from a large number of animals, ideally comparable to the population size considered in this study. This work aimed to investigate the genetic basis of CH<sub>4</sub> and carbon dioxide (CO<sub>2</sub>) emissions in Comisana dairy sheep breed using a genome-wide association study (GWAS). Gas emissions were recorded using the GreenFeed system. A total of 150 ewes were genotyped with the Infinium Ovine SNP50 v1 BeadChip (Illumina Inc., USA), which includes 58,518 markers. Quality control was performed using PLINK v1.9, and no animals were excluded based on call rate (<0.95). Single nucleotide polymorphisms (SNPs) were subsequently filtered according to call rate (>0.975), Hardy–Weinberg equilibrium ( $p < 0.00001$ ), and minor allele frequency (>0.02), resulting in a final dataset of 45,589 SNPs. Thirteen traits were analysed, including milk yield, lactose, fat and protein content, fat-and protein-corrected milk (FPCM), and CO<sub>2</sub> and CH<sub>4</sub> emissions expressed as g/d, g/FPCM, g/kg of milk fat, and g/kg of milk protein. GWAS analyses were conducted using a linear mixed model, adjusting performance data for stage of lactation (early, peak, mid, and late). A total of 11 SNPs were identified as significantly associated with the analysed traits, highlighting genes involved in epithelial cell metabolism (CSF3R, GRHL1, SLC12A2, TSPOAP1, MIPOL1 and DOCK1). These genes were associated with milk composition traits, thereby influencing CO<sub>2</sub> and CH<sub>4</sub> emissions when expressed per kilogram of milk protein or fat. These preliminary results suggest that enteric emissions may be under partial genetic control in Comisana dairy sheep.

## Session 3

## Theatre 3

### Heat stress sensitivity across lactations: The case of two Spanish dairy sheep breeds

C. Pineda-Quiroga<sup>1</sup>, I. Granado-Tajada<sup>1</sup>, E. Ugarte<sup>1</sup>, M. J. Carabaño<sup>3</sup>, M. Ramón<sup>2,3</sup>

<sup>1</sup> Neiker-BRTA, Animal Science, Campus Agroalimentario de Arkaute, 01192 Vitoria-Gasteiz, Spain, <sup>2</sup> IRIAF, Av. Del Vino s/n, 13300 Valdepeñas, Spain, <sup>3</sup> INIA-CSIC, Ctra. de la Coruña, km 7.5, 28040 Madrid, Spain

Heat stress sensitivity affects the production level in dairy cattle and varies across parities. This study estimated population thermotolerance thresholds (Thr) for the first three lactations and genetic correlations between thermotolerance indicators across lactations in Latxa (LTX) and Manchega (MNG) dairy sheep. A total of 261,389 milk records from 24,909 LTX ewes (2013–2024; 48 herds) and 493,734 records from 101,966 MNG ewes (2015–2020; 83 herds) were analyzed. Average daily temperature (Tavg) on the day of milk recording was used as the thermal load, obtained for each flock from the NASA database, based on the flock's geographical coordinates. A test-day multi-trait linear reaction norm model was applied, considering milk yield in the first three lactations as distinct traits. The model included systematic effects used in routine genetic evaluations, alongside genetic and permanent random effects. Tavg was modeling using cubic Legendre polynomials, and Thr were estimated using segmented regression analysis. Results revealed moderate Thr differences across lactations in LTX breed, ranging from 15 °C to 22 °C 17°C for the first, second, and third lactations, with production decline of about 1% per degree above Thr. In MNG, Thr ranged from 22°C to 30°C across lactations, with declines between 1% and 2% per degree. Evidence of GxE interaction was observed, being moderate in LTX and weaker in MNG breed. Genetic correlations between baseline production and the slope of decay indicated that first-lactation LTX ewes showed low to moderate sensitivity to cold, whereas, higher-producing ewes were more heat-sensitive, regardless of parity. In MNG, a moderate antagonism with cold conditions was observed across parities, while antagonism under heat conditions was considerably lower than for LTX. Finally, genetic correlations between production levels and slopes of decay across lactations, showed from negative to moderate trend with the subsequent lactation. Thus, the genetic level of heat tolerance in one lactation could condition the production level of subsequent lactations.

### Establishing a unified framework for conformation traits in dairy sheep: identification of core indicators for genetic evaluation

M. Spehar<sup>1</sup>, G. Hadjipavlou<sup>2</sup>, C. Ligda<sup>3</sup>, A. Carta<sup>4</sup>, M. Fernández<sup>5</sup>, E. Ugarte<sup>6</sup>, M. Simčič<sup>7</sup>, A. Kasap<sup>8</sup>, G. De Jong<sup>9</sup>, J. Astruc<sup>10</sup>

<sup>1</sup> Croatian Agency for Agriculture and Food, Svetošimunska cesta 25, 10000 Zagreb, Croatia, <sup>2</sup> Agricultural Research Institute, Aglantzia, 1516 Lefkosia, Cyprus, <sup>3</sup> Veterinary Research Institute ELGO-DIMITRA, Thessaloniki, Greece, <sup>4</sup> AGRIS, Loc. Bonassai Aglantzia, 07100 Sassari, Italy, <sup>5</sup> INIA-CSIC, Avenida del Vino, 13300 Valdepeñas, Spain, <sup>6</sup> NEIKER, Campus Agroalimentario de Arkaute, 01192 Arkaute, Spain, <sup>7</sup> University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia, <sup>8</sup> University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb, Croatia, <sup>9</sup> AEU CRV u.a., PO Box 454, 6800 AL Arnhem, Netherlands, <sup>10</sup> IDELE, CS52637, 31321 Castanet-Tolosan, France

Conformation traits in dairy sheep serve as relevant indicators of health, metabolic efficiency, disease resistance, reproduction ability and functional longevity. Although breeders widely recognize their importance, significant variation persist across Mediterranean breeding organizations regarding trait selection, recording methodologies, and genetic evaluation models. To enhance the simplicity and efficiency of breeding programs, it is essential to reduce and homogenize the current multitude of traits. The objective of this study was to document the existing variety of traits scored, and establish a path toward their international harmonization (development of the ICAR conformation recording guidelines). A structured survey was deployed to breeding associations, capturing population metrics and technical details of recorded traits. Responses from eight major organizations identified a diverse array of 11 frame, 7 leg, and 9 udder traits. Among these, the most frequently recorded were loin strength, rump angle, rump width, body length, and chest width (frame); foot angle, rear legs set, and fore legs set (legs); and teat position, teat length, udder depth, udder attachment, and udder cleft (udder). The identification of these common traits provides a crucial foundation for the international harmonization of conformation recording. While conformation traits may not always yield immediate economic returns, their standardization enables more precise selection for functional longevity, machine milkability, and udder health.

### Session 3

### Theatre 5

#### Genome-wide linkage disequilibrium in Pag sheep

A. Kasap<sup>1</sup>, R. Jelena<sup>1</sup>, D. Ceranac<sup>2</sup>, G. Gorjanc<sup>3</sup>, I. Pocrnić<sup>3</sup>, Z. Barać<sup>4</sup>, V. Držaić<sup>1</sup>, B. Mioč<sup>1</sup>, D. Jurković<sup>2</sup>, D. Barać<sup>2</sup>, Z. Ivkić<sup>2</sup>, M. Špehar<sup>2</sup>

<sup>1</sup> University of Zagreb Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia, <sup>2</sup> Croatian Agency for Agriculture and Food, Svetošimunska 25, 10000 Zagreb, Croatia, <sup>3</sup> University of Edinburgh, Roslin Institute, Easter Bush, EH25 9RG Edinburgh, United Kingdom, <sup>4</sup> Croatian Association of sheep and goat breeders, Jarnovičeva 17c, 10000 Zagreb, Croatia

**Summary** The objective of this study was to characterize the extent of linkage disequilibrium (LD) at various physical distances across all 26 autosomes in Pag sheep. A total of 2,637 sheep (113 ♂ and 2,524 ♀) were genotyped using the Illumina Infinium Ovine SNP50 v1 BeadChip. After updating map files to the Oar\_v3.1 reference genome and applying QC (animal call rate > 0.90, SNP call rate > 0.95), 42,039 autosomal SNPs were retained for analysis. The LD statistic  $r^2$  was estimated within a 1 Mb sliding window using PLINK v1.9, involving a total of 714,649 SNP pairs. The analysis of LD decay revealed relatively high genetic linkage at short physical distances. In the initial interval of 0-100 kb, the mean  $r^2$  was 0.080. As expected, LD declined rapidly as physical distance increased, dropping to 0.029 in the 100-200 kb range and reaching a baseline of approximately 0.009 at the 1 Mb threshold. This sharp decline indicates that the population has maintained sufficient historical recombination to break down long-range associations, which is a key indicator of “healthy” genetic diversity. Chromosome (CHR) -specific LD generally followed the expected decay pattern relative to chromosome length, while CHR 10 emerged as a significant exception with an  $r^2$  of 0.027. This atypical LD level is particularly striking because CHR 10, with only 26,715 marker pairs, shows higher linkage than much larger autosomes, such as CHR 1 (81,672 pairs;  $r^2 = 0.020$ ). Such localized LD peaks are indicative of selective sweeps, suggesting that CHR 10 may harbour key genes for breed-specific adaptations or specialized production traits, such as milk yield and composition, which have been favoured by selective breeding. However, further investigation through pathway analysis is required to elucidate the specific genes underpinning the signatures of selection in this breed. **Key words:** Pag sheep, LD, genetic diversity, chromosome

### Using daily data from automated milking systems provides larger estimates of heat stress effects than data from traditional monthly milk recording

E. Reinoso<sup>1</sup>, M. J. Carabaño<sup>1</sup>, C. Díaz<sup>1</sup>, M. Ramón<sup>1</sup>

<sup>1</sup> INIA-CSIC, Animal Breeding and Genetics, Ctra. de La Coruña km 7.5, 28040 Madrid, Spain

Official milk recording information obtained at a monthly pace is currently used for genetic evaluations of heat tolerance (HT) in dairy cattle. However, higher resolution information may be needed to properly capture heat stress (HS) effects. This study aimed at comparing the use of official monthly recording (MR) vs. daily records (DR) from milking robots to determine HS effects and genetic parameters related with HT of cows. After edits, 11.7 DR and 0.35 MR million records of milk (M), fat (F) and protein (P) yields on the day of recording from 27 thousand Holstein-Friesian cows in 213 herds in Spain in years 2020-25 were used in the analyses. Ambient temperatures and relative humidity were obtained from the NASA POWER repository and used to calculate the temperature-humidity index (THI) for cattle. Population heat tolerance thresholds (Thr) and slopes of production loss under HS (Slp) were obtained using a linear model including effects of herd-year of calving, lactation-age at calving, lactation-stage of lactation and a cubic Legendre polynomial as fixed effects and the cow effect as random effect. A segmented line approach with one change point was then fitted to the cubic polynomial to obtain the desired parameters. Estimates for Thr were similar for MR and DR data, ranging from average daily values of 18°C or THI=67 for P yield to 24 °C or THI=72 for M yield. On the other hand, Slp estimates were larger for DR data (240 and 5 g/d, °C for M and F, P yields, respectively) than for MR data (150 and 3 g/d, °C for M and F, P yields, respectively). The estimation of individual genetic components for HT (Slp\_g) and associated genetic parameters were obtained from random regressions on heat loads for individual animals. DR data detected much larger individual losses, with maximum values of 970/40/48 g/d, °C for M/F/P than MR data (maximum 280/10/10 g/d, °C for M/F/P). Estimates of GxE interactions for DR data were also larger than for MR data. Pearson correlations of EBVs for HT from DR and MR data ranged from moderate for M to low for F and P yields. Overall, as expected, DR information captured larger effects of HS and HT differences among cows than MR data.

## Session 3

## Theatre 8

### Toward an objective typology of Sardinian dairy-sheep farms: a reproducible multivariate pipeline with clustering model selection

M. G. Serra<sup>1</sup>, M. Acciaro<sup>1</sup>, M. Decandia<sup>1</sup>, V. Giovanetti<sup>1</sup>, R. Fadda<sup>2</sup>, S. Throude<sup>3</sup>

<sup>1</sup> AGRIS, Animal production, Loc. Bonassai S.S. 291 Sassari-Fertilia - Km. 18,600 - Sassari, 07100 Sassari, Italy, <sup>2</sup> LAORE, Via Caprera n. 8, 09123 Cagliari, Italy, <sup>3</sup> IDELE, 149 rue de Bercy, 75012 Paris, France

We propose and test a reproducible workflow to typologize Sardinian dairy-sheep farms (partners of LIFE Green-sheep project) into management types (extensive, semi-extensive, semi-intensive, intensive) using farm indicators chosen for availability, data quality, and substantive relevance. Continuous variables describing feeding inputs, land use, stocking rate, and milk yield were standardized; univariate outliers were excluded; highly collinear pairs ( $|r| \geq 0.60$ ) were pruned, retaining one indicator in each pair. Sampling adequacy supported dimensionality reduction (overall measure of sampling adequacy, KMO  $\approx 0.62$ ; Bartlett's test significant). We then applied a two-stage approach: (i) a first PCA to decide the number of components and screen out redundant variables, followed by (ii) a second PCA with Varimax (Kaiser normalization) to obtain orthogonal rotated components (RCs) and scores. On these RCs and, in parallel, on pruned standardized raw features, we compared multiple clustering strategies: hierarchical agglomerative methods (average, complete, single, Ward.D2; plus AGNES and DIANA), k-means (multiple starts), and PAM on Euclidean distances. For each dataset/method and candidate number of clusters (k), we computed internal validation indices: mean silhouette width, Dunn and Dunn2, Calinski-Harabasz, Davies-Bouldin, S\_Dbw, and (for hierarchical fits) cophenetic correlation. Models were ranked via a composite mean-rank across indices. The top combination was agglomerative hierarchical clustering (AGNES, average linkage/UPGMA) on Euclidean distances computed from varimax-rotated factor scores; the tree was cut at  $k = 4$ . Cluster labels were mapped back to the original data, and a 2D RC1-RC2 plot with robust 95% ellipses showed a clear separation for three groups plus a smaller, more extreme fourth group. Early analyses of the untransformed variables corroborate the separation among clusters, reinforcing the validity of the proposed grouping.

### **The influence of breeding values obtained through morphofunctional assessment on lifetime production and productive longevity in Murciano-Granadina goats**

J. V. Delgado Bermejo<sup>1</sup>, J. M. León Jurado<sup>2</sup>, J. I. Salgado Pardo<sup>1</sup>, J. Fernández Álvarez<sup>3</sup>, A. González Ariza<sup>2</sup>

<sup>1</sup> University of Córdoba, Genetics Department, C5 Building, Rabanales University Campus, 14071 Córdoba, Spain, <sup>2</sup> Diputación of Córdoba, Agropecuario Provincial Center, N-IV road, Km.396, 14071 Córdoba, Spain, <sup>3</sup> National Association of Breeders of Murciano-Granadina Goat Breed, GR-3405 road, 18340 Fuente Vaqueros, Spain

The Murciano-Granadina goat is the most significant Spanish breed in the dairy goat sector and faces a growing need promote its genetic improvement while preserving resilience traits, such as lifetime production rate and productive longevity. To this goal, a tool has been developed to allow the identification of morphofunctional variables that carry the most weight in discriminating between groups of animals with different lifetime production and productive longevity. The values of 17 variables (stature, chest width, body depth, rump width, rump angle, angulosity, bone quality, anterior insertion, rear insertion height, median suspensor ligament, udder width, udder depth, nipple placement, nipple diameter, rear legs rear view, rear legs side view, and mobility) were obtained through linear grading of 8,391 goats, and 10 groups were generated according to the deciles for the variables lifetime production, on the one hand, and productive longevity on the other. The breeding values of the variables included in the “Mammary System” macro-area (‘nipple diameter’, ‘udder depth’, and ‘nipple placement’ variables) showed the most discriminatory weight when differentiating animals into lifetime production-based groups. According to their productive longevity, the variables ‘nipple diameter’, ‘rump angle’, and ‘udder depth’ showed the most discriminatory power. Animals with more positive breeding values for udder depth and nipple diameter also showed the most desirable values for lifetime production and productive longevity traits. However, the breeding values of ‘legs aplomb’ showed a reduced impact in the statistical analysis. Therefore, results suggest that a reduction of zoometric evaluation procedures is possible, as they are not only able to describe the status of a certain population but can also be used to predict the future evolution of parameters.

### Session 3

### Theatre 10

### **Sperm and blood epigenetic diversity in local and cosmopolitan Italian cattle breeds**

E. Capra<sup>1</sup>, R. Negrini<sup>2</sup>, B. Lazzari<sup>1,2</sup>, P. Cozzi<sup>1</sup>, A. Tafuri<sup>1</sup>, P. Ajmone Marsan<sup>2</sup>, A. Stella<sup>1</sup>, F. Turri<sup>1</sup>

<sup>1</sup> National Research Council (CNR), Institute of Agricultural Biology and Biotechnology (IBBA), Via Alfonso Corti nr. 12,, 20133 Milano, Italy, <sup>2</sup> Università Cattolica del Sacro Cuore, Department of Animal Science, Food and Nutrition – DIANA, Via Emilia Parmense 84, 29122 Piacenza, Italy

Our group recently characterized breed-specific epigenetic diversity in cattle blood (Angus vs Nellore) and in sperm from Holstein and Montbéliarde breeds. We observed that breed-related epigenetic variation is partially consistent between somatic and germinal tissues and may be linked to embryonic development and quantitative traits. To further investigate bovine epigenetic biodiversity, we analyzed DNA methylation profiles from several Italian autochthonous and cosmopolitan cattle breeds in both somatic (blood) and germinal (sperm) tissues. A wide epigenetic characterization of 80 bulls across 11 breeds with different productive purposes, specifically dairy (7 Frisona, 7 Pezzata Rossa), dual-purpose (4 Modenese, 6 Valdostana Pezzata Rossa, 6 Varzese), and beef (6 Blu Belga, 10 Chianina, 10 Marchigiana, 10 Piemontese, 4 Podolica, 10 Romagnola) was obtained. Blood and sperm were analyzed using Reduced Representation Bisulfite Sequencing (RRBS). In addition, whole-genome sequencing (WGS) was performed on pooled samples from multiple individuals within each breed. RRBS data were analysed using nf-core. The *Bos taurus* ARS-UCD1.2 genome was used as the reference. Methylation calls were extracted with Bismark. SeqMonk software was used for visualization and calculation of methylation variation between samples. Overall, 31 billion reads were generated, with an average of approximately 182 million reads (55Gb) per sample. Preliminary results revealed distinct tissue specific CpG methylation patterns in blood and sperm. Ongoing analyses aim at evaluating breed-related epigenetic diversity in both somatic and germline cells. This work will provide an epigenetic map of Italian cattle, highlighting potential heritable methylation patterns and providing insights into how genetic and epigenetic variations contribute to breed differentiation and adaptation, also affecting functional traits relevant to beef and dairy production.

### Runs of homozygosity for the detection of putative selection signatures in the Lesvos dairy sheep breed

A. L. Hager-Theodorides<sup>1</sup>, A. Kominakis<sup>1</sup>, I. Mastranestasis<sup>2</sup>, G. Mészáros<sup>3</sup>

<sup>1</sup> Agricultural University of Athens, Animal Science, Iera Odos 75, 11855 Athens, Greece, <sup>2</sup> Breeder's Association of the Lesvos sheep, Anaxos, 81109 Lesvos, Greece, <sup>3</sup> BOKU University of Natural Resources and Life Sciences, Institute of Livestock Sciences, Gregor-Mendel-Straße 33, 1180 Wien, Austria

Lesvos sheep is a dairy breed originating from the homonymous island, adapted to semi-arid conditions and traditionally managed under semi-extensive rearing systems. In this study we employed runs of homozygosity (ROH) analysis to identify genomic regions with elevated homozygosity that putatively reflect past selection and/or demographic pressure. Genotypes from 1,144 animals assayed with the Illumina ovine SNP50 beadchip array were subjected to quality control (individual sample and SNP with call rate < 0.90, SNPs with HWE  $P < 1 \times 10^{-9}$  excluded) and all 49,323 SNP autosomal markers were retained for subsequent analysis. ROH were identified in PLINK (-homozyg) using a minimum length of 1 Mb, minimum 15 SNPs, and allowing 0 heterozygous and 0 missing calls per window. ROH islands were defined as genomic regions containing SNPs in the top 0.1% for ROH incidence across animals. We identified three ROH islands, two on chromosome 2 (0.98 and 1.13 Mb) and one on chromosome 5 (0.88 Mb). Twelve genes were located within these genomic regions including candidates involved in cellular stress responses (EGR1, HSPA9), male fertility (SPAG8, CCIN) and muscle function (TPM2). These results suggest that the ROH-enriched regions identified here are consistent with putative selection related to environmental resilience and performance under semi-extensive management and provide gene and genomic region candidates for further studies.

### Session 3

### Poster 12

### Genomic assessment of scrapie resistance, genetic diversity, and inbreeding in three indigenous sheep breeds of Epirus (Greece)

A. Kominakis<sup>1</sup>, M. Goliomytis<sup>1</sup>, D. Gelia<sup>2</sup>, D. Pappas<sup>3</sup>, A. L. Hager-Theodorides<sup>1</sup>

<sup>1</sup> Agricultural University of Athens, Department of Animal Science, Iera Odos 75, 11855 Athens, Greece, <sup>2</sup> Association of Pastoral Farmers of Epirus, Ethnikis Antistaseos Street 5, 45221 Ioannina, Greece, <sup>3</sup> Ioannina Center for Animal Genetic Resources, Panepistimiou Street, Terma, 45110 Katsika Ioannina, Greece

The present study reports the genomic characterization of three indigenous Greek sheep breeds (Kalarrtyiki, Katsika and Oreino) of Epirus region. A total of 300 animals (100 per breed) were genotyped using the 63K Ovine SNP array. The objectives of the study were to: (i) determine prion protein (PRNP) genotypes and classify animals into scrapie resistance categories; (ii) estimate observed heterozygosity at individual and breed levels; and (iii) quantify genomic inbreeding using runs of homozygosity (ROH)-based coefficients across different temporal scales. ROHs were identified using a sliding window approach, defining a ROH as  $\geq 30$  consecutive SNPs spanning  $\geq 1$  Mb. These segments were used to estimate individual FROH and to partition autozygosity into length classes reflecting recent versus ancient inbreeding. PRNP genotypes were inferred from polymorphisms at codons 136, 154 and 171, and animals were assigned to five resistance classes. Kalarrtyiki and Katsika exhibited a high prevalence ( $>80\%$ ) of resistant genotypes (classes 1–2), indicating no immediate need for scrapie-related intervention. In contrast, Oreino showed a higher proportion of moderately susceptible animals and included a few highly susceptible individuals, warranting targeted management. ROH-based inbreeding coefficients revealed clear breed differences: Kalarrtyiki displayed the highest mean inbreeding and reduced heterozygosity, with evidence of recent autozygosity, whereas Katsika and Oreino showed lower average inbreeding and greater variability. A strong negative correlation was observed between heterozygosity and inbreeding. Notably, highly resistant genotypes were associated with elevated inbreeding levels, highlighting the risks of unilateral selection. Overall, the findings support balanced breeding strategies integrating disease resistance with long-term genetic diversity conservation in small local breeds.

### Phenotypic data to assess resilience of Sicilian horse breeds in the context of climate change

M. Carlentini<sup>1</sup>, S. Tumino<sup>1,2</sup>, A. Antoci<sup>1</sup>, G. Chessari<sup>1</sup>, A. Criscione<sup>1</sup>, S. Bordonaro<sup>1,2</sup>, D. Marletta<sup>1,2</sup>

<sup>1</sup> University of Catania, via Santa Sofia, 95123 Catania, Italy, <sup>2</sup> Centro A.SI.ZO.CA.CLI., via Santa Sofia, 95123 Catania, Italy

The two Sicilian horse breeds, Sanfratellano (SAN) and Purosangue Orientale (PSO), are renowned for their adaptability to Mediterranean conditions. The study aimed to assess equine welfare under heat-stress conditions by analysing physiological and haematological responses. A total of 15 PSO and 6 SAN was monitored during thermotolerance TT and heat-stress HS seasons. Overall 13,124 post-QC Heart rate (HR) and Skin Temperature (SKT) records were recorded using wearable IoT sensors hourly for 15 days per season. Blood samples were collected at the beginning and end of each season (84 samples). A Linear Mixed Model (LMM) for repeated measures was used to analyse phenotypic data including breed, season, and breed-season interaction as fixed effects and Temperature Humidity Index THI as a covariate. HR was significantly influenced by breed, with the PSO exhibiting a higher value than the SAN. SKT showed significant differences across breed, season, and their interaction. Notably, the SAN presented higher mean value than the PSO in the HS, but lower value in the TT. White blood cell (WBC), neutrophil and lymphocyte counts were significantly higher in the HS than in TT, reflecting seasonal immune responses. PSO subjects exhibited lower WBC, eosinophil, and basophil counts than SAN during the HS period. The increase in erythrocytes (RBC) during the HS season compared with the TT season, probably related to adaptation aimed at improving oxygen transport during thermal stress. In the breed-season interaction, the SAN showed lower RBC, haemoglobin and haematocrit values than the PSO under HS condition. The T3 thyroid hormone concentration, was significantly lower in the HS season than the TT one, indicating an adaptive response to reduce metabolic heat load. This first batch of data confirms the complex physiological response of these two horse breeds to thermal stress, enabling better evaluation of their physiological adaptability in challenging environments. This research was funded by PRIN 2022 “Equine resilience and welfare in Climate change and stressful scenarios using Omic technologies and innovative low cost Sensors –ECOS”.

## Session 3

## Poster 14

### Metabolic indicators of heat-tolerant phenotypes in cattle: insights from blood metabolomics of Angus and Nellore under natural heat stress

G. Rocchetti<sup>1</sup>, E. Capra<sup>2</sup>, M. Errico<sup>1</sup>, L. Lucini<sup>3</sup>, J. F. Garcia<sup>4</sup>, G. De Paula Nogueira<sup>4</sup>, Y. T. Utsunomyia<sup>4</sup>, A. M. Perez O'Brian<sup>4</sup>, M. Milanese<sup>1</sup>, P. Ajmone Marsan<sup>1</sup>

<sup>1</sup> Università Cattolica del S. Cuore, Animal Science Food and Nutrition - DIANA, via E. Parmense, 86, 29122 Piacenza, Italy, <sup>2</sup> National Research Center -CNR, Institute of Agricultural Biology and Biotechnology, National Research Council IBBA, via A. Corti, 12, 20133 Milano, Italy, <sup>3</sup> Università Cattolica del S. Cuore, Dipartimento di Scienze e Tecnologie Alimentari per una filiera agro-alimentare Sostenibile - DISTAS, via E. Parmense, 86, 29122 Piacenza, Italy, <sup>4</sup> São Paulo State University, Faculdade de Medicina Veterinária de Araçatuba, R. Clóvis Pestana, 793, 16010 Araçatuba, Brazil

Angus (*Bos taurus*) and Nellore (*Bos indicus*) have opposite genetic backgrounds for thermotolerance and are good models to identify metabolic traits linked to resilience. Using untargeted blood metabolomics, we characterized breed-specific responses to natural heat stress (HS) and identified potential physiological indicators for breeding. Blood samples from five sun-exposed animals per breed were collected in February (HS) and June (cool season). Profiles were obtained by LC–Orbitrap mass spectrometry and analyzed using multivariate methods and KEGG pathway analysis. Under HS, a clear breed separation emerged. Nellore showed efficient energy management and limited accumulation of stress-related intermediates, whereas Angus exhibited increased lipid mobilization and mitochondrial load, reflected by higher acyl-carnitines and lysophospholipids. Comparisons between HS and post-HS periods revealed consistent changes in amino acids, phospholipids, and nucleotide derivatives, often with opposite trends. Among 22 shared discriminating metabolites, seven phospholipid-related compounds were significantly higher in Angus under HS ( $p < 0.05$ ; cumulative Log2FC = 3.73) than in Nellore (−7.95), supporting differences in membrane dynamics and oxidative balance. Pathway analysis highlighted purine and porphyrin metabolism as common components of the HS response. The stronger reduction of 5-aminoimidazole ribotide and cAMP in Nellore suggested more efficient stress-regulatory mechanisms. Overall, thermotolerant cattle appear to minimize the energetic cost of homeostasis during heat exposure.

### Characterization of enteric methane emissions in autochthonous Spanish dairy sheep breeds

*I. Granado-Tajada*<sup>1</sup>, *C. Pineda-Quiroga*<sup>1</sup>, *J. Valdés-Hernández*<sup>1</sup>, *I. Goiri*<sup>1</sup>, *H. Benhissi*<sup>1</sup>, *M. Medjadbi*<sup>1</sup>, *E. Ugarte*<sup>1</sup>, *A. García-Rodríguez*<sup>1</sup>, *A. Rubio-Juan*<sup>3</sup>, *G. Cañadas*<sup>3</sup>, *M. Ramón*<sup>2</sup>

<sup>1</sup> NEIKER\_BRTA, N-104, km. 355, 01192 Arcaute, Spain, <sup>2</sup> INIA-CSIC, Mejora Genética Animal, Crta A Coruña km 7,5, 28040 Madrid, Spain, <sup>3</sup> CERSYRA-IRIAF, Av. del Vino, 13300 Valdepeñas, Spain

Small ruminants are responsible for 7% GHG emissions from livestock farming, mainly attributed to enteric methane (CH<sub>4</sub>). Genetic selection of low-CH<sub>4</sub> emitter animals is a promising mitigation strategy since it is permanent and cumulative over time. The objective of this work was to estimate genetic components for CH<sub>4</sub> traits in Manchega (MNG) and Latxa (LTX) autochthonous dairy sheep breeds. Data on CH<sub>4</sub> emission were taken three times/week, one week/month for 5 months on MNG (N=96) and LTX (N=144) experimental herds. A total of 1375 MNG and 2136 LTX records were collected using a portable non-dispersive infrared CH<sub>4</sub> detector. The following phenotypes were calculated on monthly basis: CH<sub>4</sub> concentration (ppm), CH<sub>4</sub>/CO<sub>2</sub> ratio, CH<sub>4</sub> production (g/day), and CH<sub>4</sub> intensity (g/milk yield). Variance components for CH<sub>4</sub> traits and their genetic correlations with production and functional traits were estimated. Phenotypic means were 470 and 1340 ppm, 53 and 55 g/day, 29 and 28 g/milk yield, and 0.07 and 0.09 CH<sub>4</sub>/CO<sub>2</sub> ratio, for MNG and LTX respectively. Estimated heritabilities were low to moderate, ranging from 0.04 to 0.08 for MNG and 0.05 to 0.12 for LTX, being higher for CH<sub>4</sub> production. Repeatability was estimated between 0.08 and 0.15 for MNG and 0.15 and 0.21 for LTX, suggesting a notable environment influence on these traits. Correlations between CH<sub>4</sub> traits were high except for CH<sub>4</sub> concentration. Correlation between CH<sub>4</sub> traits with production and functional traits were low to moderate in both breeds. CH<sub>4</sub> concentration was negatively correlated with production traits (up to -0.19 in MNG). For the other CH<sub>4</sub> traits, correlations were positive and low, between 0.02 and 0.15. These results are of interest for the development of breeding strategies aimed at mitigating greenhouse gas emissions in these breeds. The negative genetic correlation observed between CH<sub>4</sub> concentration and productive traits makes the development of such strategies more challenging as it is important to ensure long-term productive sustainability

## Session 4

## Theatre 2

### Precision reproductive management as an alternative to synchronisation protocols in dairy farms

*L. Zambianchi*<sup>1</sup>, *B. Merlo*<sup>1</sup>, *E. Giaretta*<sup>2</sup>, *I. Fusaro*<sup>3</sup>, *R. Colleluori*<sup>1</sup>, *A. Formigoni*<sup>1</sup>, *L. Mammi*<sup>1</sup>

<sup>1</sup> University of Bologna, Via Tolara di Sopra 50, 40064 Ozzano Emilia, Italy, <sup>2</sup> University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy, <sup>3</sup> University of Teramo, Piazza A. Moro 45, 64100 Teramo, Italy

Reproductive efficiency is determinant for productivity and sustainability in dairy cow farming and remains one of the main causes of involuntary culling. Hormonal estrous synchronization protocols are widely used to optimize insemination timing and work organization. Increasing concerns related to animal welfare have stimulated interest in alternative reproductive management strategies that reduce the routine use of exogenous hormones. This study aimed to compare two reproductive management approaches under commercial dairy farming conditions: a Double-Ovsynch synchronization protocol (SYNCH) and a precision-based strategy (PREC) relying on natural heat detection through automated monitoring systems. The trial was conducted in two medium-sized farms, where 137 multiparous cows (PREC: 66, SYNCH: 71) were monitored from dry-off until the second AI. At calving, animals were randomly assigned to one of the two reproductive strategies. Reproductive and health data were recorded at the individual level. Reproductive performances were statistically analysed by mixed model (JMP Pro v.19). The relative risk of pregnancy (PREC vs SYNCH) was 1.31, with no significant difference for the two protocols ( $p > 0.05$ ). Conception rate did not differ between the two protocols (PREC: 29.82%; SYNCH: 23.73%) at both first (PREC: 27.3%; SYNCH: 23.9%) and second (PREC: 35.4%; SYNCH: 25.5%) insemination ( $p > 0.05$ ). A significant treatment effect on heat detection rate (HDR) was observed ( $p < 0.001$ ), with SYNCH cows showing a reduced HDR (9.09%) compared with PREC cows (90.32%). Further investigation on progesterone level at AI will clarify whether the lack of detected heat in SYNCH cows reflects silent estrus or failure of synchronization. These results show that precision-based reproductive strategy represent a valid and effective alternative to systematic hormonal synchronization in medium-sized dairy farms, and its wider adoption could contribute to a further reduction in hormone use without compromising fertility performance. Funded by European Union, Next Generation EU.

### Can mid-infrared (MIR) spectral data from milk be used to select more fertile sheep prior to artificial insemination?

I. Beltrán De Heredia<sup>1</sup>, J. Valdes Hernandez<sup>1</sup>

<sup>1</sup> NEIKER-BRTA, Animal Science, Campus agroalimentario N 104, Km 355, 01192 Arcaute, Spain

Artificial insemination (AI) in sheep is limited by relatively low fertility rates. The aim of this study was to objectively and a priori identify ewes with higher fertility. The study included 206 Latxa ewes inseminated on 29 July 2024 during the milking period. Milk samples were collected on 24 June 2024, prior to AI, and analyzed using mid-infrared (MIR) spectroscopy. Additional variables were recorded, including ewe age, previous lambing interval (LII), number of lambings (NL), parity, days in milk, milk yield (MY), and body condition score at milk control and after AI. Lambing dates, number of lambs born, and stillbirths were also recorded. Flock fertility was 44%, with a prolificacy rate of 1.54 and lamb mortality at birth of 2.86%. Raw MIR spectra were cleaned by removing outliers, noisy regions, and baseline areas, and were pretreated using a Savitzky–Golay filter and first-derivative approach. The pretreated MIR dataset (N = 525 bands) and fertility outcome (binary: 0 or 1) were analyzed using PLS-DA, including parameter tuning and model assessment (accuracy, sensitivity, and specificity). Spectral bands were preselected based on a VIP score  $\geq 0.80$ , non-zero regression coefficients, and a selection frequency  $\geq 80\%$  across models. The effect of each selected MIR band (N = 433), together with other relevant variables (e.g., age, NL, LII, MY, and parity), was assessed using a multivariable logistic regression model. Significant MIR bands (N = 20;  $P < 0.05$ ) were subsequently used in a second PLS-DA model to predict divergent fertility groups. Pre-AI milk MIR spectra showed limited predictive ability for fertility (accuracy = 0.55). However, they were useful in discriminating between ewes with divergent fertility levels (high fertility: accuracy = 0.92, specificity = 0.81, sensitivity = 0.96; low fertility: accuracy = 0.92, specificity = 0.96, sensitivity = 0.81). Applying these findings would allow exclusion of low-fertility ewes from AI, increasing fertility among inseminated animals to 48% vs 44%. Also, discarded ewes (16% of the flock) would show a fertility rate of 24% ( $P = 0.05$ ). These results could be applied to the total ewes inseminated during the milking period, in order to confirm the usefulness of the study.

### Session 4

### Theatre 4

#### Effects of intramammary infections and dry-off treatment on circulating leukocytes populations of Alpine dairy goats around dry-off

M. Mezzetti<sup>1</sup>, A. Grassi<sup>1</sup>, H. T. Bosi<sup>1</sup>, S. Scemama De Gialluly<sup>1</sup>, M. Sfulcini<sup>1</sup>, M. M. Vignati<sup>2</sup>, A. Vercesi<sup>1</sup>, F. Amadeo<sup>1</sup>, R. Cantarini<sup>1</sup>, M. F. Addis<sup>2</sup>, A. Minuti<sup>1</sup>, F. Piccioli Cappelli<sup>1</sup>, S. Fasana<sup>3</sup>, P. Moroni<sup>2</sup>, E. Trevisi<sup>1</sup>

<sup>1</sup> Università Cattolica del Sacro Cuore, Department of Animal Sciences, Food and Nutrition (DIANA), Via Emilia Parmense 84, 29122 Piacenza, Italy, <sup>2</sup> Università degli Studi di Milano, Department of Veterinary Medicine and Animal Sciences (DIVAS), Via dell'Università, 6, 26900 Lodi, Italy, <sup>3</sup> Freelance goat veterinary practitioner, Via Damiano Chiesa, 7, 22100 Como, Italy

Seventy-nine Alpine goats were enrolled 7 days before dry-off (DFD) in a  $2 \times 2$  experimental design. Milk samples were analyzed by bacterial culture and isolates identified by MALDI-TOF. Goats were classified according to udder health status (UH) as healthy (HEAL: sterile samples in both halves;  $n = 45$ ) or infected (INFE: at least one positive half;  $n = 34$ ). At dry-off, goats were blocked by previous lactation milk yield, SCC and BCS; in INFE goats, the number of positive halves and bacterial species were also considered. Goats were abruptly dried off without treatment (control group, CG;  $n = 41$ ) or treated with an intramammary cephalosporin suspension (treatment group, TG;  $n = 38$ ). Blood samples were collected at dry-off (before treatment) and at 7 DFD to assess hematological parameters. Data were analyzed using a mixed model for repeated measures including UH, antibiotic treatment, time and their interactions as fixed effects, with parity as a covariate. Compared with HEAL, INFE goats showed higher red blood cell count, eosinophil percentage, hematocrit and red blood cell distribution width, and lower mean corpuscular hemoglobin concentration throughout the study ( $p \leq 0.05$ ). At 7 DFD, INFE goats had lower mean corpuscular hemoglobin ( $p = 0.01$ ) and platelet counts than HEAL goats ( $p = 0.06$ ). Compared with CG, TG goats showed a lower neutrophil percentage ( $p = 0.07$ ) and a greater eosinophil percentage at 7 DFD ( $p < 0.01$ ). In HEAL goats, TG had a higher basophil percentage at 7 DFD than CG ( $p = 0.06$ ). These results indicate that udder health status influences hematological and leukocyte profiles around dry-off, and that intramammary antibiotic treatment at dry-off modulates circulating leukocyte populations in the early dry period.

### Impact of low concentrations of DDT and HCB on survival and apoptosis of bovine mammary epithelial cells

J. Para<sup>1</sup>, M. Gajewska<sup>2</sup>, K. Meade<sup>3</sup>, A. Zachwieja<sup>1</sup>, A. E. Zielak-Steciwo<sup>1</sup>

<sup>1</sup> Wrocław University of Environmental and Life Sciences, Institute of Animal Breeding, Chelmońskiego 38c, 51-630 Wrocław, Poland, <sup>2</sup> Warsaw University of Life Sciences, Department of Physiological Sciences, Nowoursynowska 159b, 02-776 Warszawa, Poland, <sup>3</sup> University College Dublin, School of Agriculture and Food Science, Belfield, 4 Dublin, Ireland

According to EFSA reports (2009-2022), the most frequently detected pesticide residues in cow's milk in Europe are DDT and hexachlorobenzene (HCB). DDT is an organochlorine insecticide formerly used to control malaria-transmitting mosquitoes and has been banned in most countries since the 1970s. HCB, a highly persistent fungicide, was prohibited in 2001 under the Stockholm Convention. Both compounds are highly persistent, bioaccumulative and toxic. Although DDT and HCB have been banned in Europe for decades, their residues persist in the environment and continue to be detected in food products, including milk. Despite this widespread exposure, the direct effects of chronic low-dose contact with these compounds on the function of bovine mammary epithelial cells (BME) remain poorly understood. Therefore, the aim of this study was to investigate the effects of low environmental concentrations of selected pesticides on the viability of BME cells. An experiment was performed using established bovine mammary epithelial cell line BME-UV1, maintained in DMEM/F-12 medium supplemented with 10% FBS, insulin, holo-transferrin and antibiotics under standard culture conditions. DDT and HCB were administered individually at concentrations of 0.01-10 µM and 0.01-5 µM, respectively. Following 24h of exposure, cellular viability was evaluated using the MTT assay, while the proportion of apoptotic cells was determined by annexin V assay. Exposure of BME-UV1 cells to DDT and HCB induced moderate cytotoxicity and apoptosis. HCB caused a more pronounced reduction in cell viability and stronger apoptosis at higher concentrations (1-5 µM). In contrast, DDT exhibited a non-linear dose-response relationship, with the lowest concentrations (0.01-0.1 µM) inducing a greater decrease in viability and stronger apoptotic effect than higher doses. Further analyses will enable a more comprehensive understanding of the molecular basis of these effects.

### Time trends and diagnostic markers reflecting the prevalence of intramammary infections at dry-off in Alpine dairy goats

M. Mezzetti<sup>1</sup>, A. Grassi<sup>1</sup>, H. T. Bosi<sup>1</sup>, S. Scemama De Gialluly<sup>1</sup>, M. Sfulcini<sup>1</sup>, M. M. Vignati<sup>2</sup>, A. Vercesi<sup>1</sup>, F. Amadeo<sup>1</sup>, R. Cantarini<sup>1</sup>, M. F. Addis<sup>2</sup>, A. Minuti<sup>1</sup>, F. Piccioli Cappelli<sup>1</sup>, S. Fasana<sup>3</sup>, P. Moroni<sup>2</sup>, E. Trevisi<sup>1</sup>

<sup>1</sup> Università Cattolica del Sacro Cuore, Department of Animal Sciences, Food and Nutritio (DIANA), Via Emilia Parmense 84, 29122 Piacenza, Italy, <sup>2</sup> Università degli Studi di Milano, Department of Veterinary Medicine and Animal Sciences (DIVAS), Via dell'Università 6, 26900 Lodi, Italy, <sup>3</sup> Freelance goat veterinary practitioner, Via Damiano Chiesa, 22100 Como, Italy

Half udder milk samples were collected from seventy-nine Alpine goats at -7 days from dry-off (DFD) and 0 DFD. Samples were analyzed by bacterial culture and isolates identified by MALDI-TOF. Changes in intramammary infection (IMI) prevalence over time were evaluated using generalized linear mixed models with binomial distribution, including random intercepts for goat and half-udder. Infection dynamics were further investigated by estimating probabilities of new IMI and clearance between -7 and 0 DFD using intercept-only binomial mixed models with random goat effects. Results are presented as estimated probabilities or odds ratios with 95% CI. Temporal changes in pathogen categories were evaluated using separate binomial mixed models. Further, SCC was measured and California mastitis test (CMT) was performed, and data were analyzed using linear mixed models and binomial GLMM, respectively, to assess the effect of time, udder health status and their interaction, accounting for clustering within goats and operators when appropriate. The odds of IMI were higher at 0 than at -7 DFD (OR = 2.06; 95% CI: 1.45-2.92;  $p < 0.01$ ), whereas odds of major pathogens, non-aureus staphylococci and mammaliococci, and other bacteria did not change. The estimated probability of IMI clearance was 38% (95% CI: 22-57%), while the probability of new IMI was 3.5% (95% CI: 1.3-9.2%). SCC increased over time ( $p < 0.01$ ) and was higher in positive than negative udders ( $p < 0.01$ ). No effects of time or udder health status were observed for CMT.

**Restricted social contact during gestation affects reproductive performance of sows**P. Seddaiu<sup>1</sup>, K. Barłowska<sup>1</sup>, I. Camerlink<sup>1</sup><sup>1</sup> Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Postępu 36A, 05-552 Jastrzebiec, Poland

Social relationships are an important aspect of health and welfare and may be particularly relevant during pregnancy. In farm animals, gestating females face various social stressors, including limited social contact, which might affect reproductive success. This study investigated the effect of restricted social contact during gestation on the behaviour and reproductive outcomes of sows. For one month during gestation, 31 Puławska gilts were assigned to one of three treatment groups: housed alone, housed alone with 3 h/day of social contact, or group-housed with full contact. Farrowing ease, nursing ability, and maternal behaviour were scored on a 3-point scale (good, moderate or bad). Data included also the number of liveborn, stillborn, growth-retarded piglets (IUGR-Intrauterine Growth Restriction), mummies, piglet mortality and average daily gain (ADG) between 5-28 days of life. Data were analysed using mixed models and piglet survival was analysed using Kaplan-Meier methods. Group-housed sows had better scores for farrowing ease than sows housed alone ( $z = 2.12$ ,  $p = 0.03$ ). Social treatment did not affect the number of liveborn piglets ( $p = 0.17$ ). Occurrence of mummies (1.88%), stillborn (1.88%) and IUGR piglets (3.75%) was low. Most sows showed a good nursing ability and maternal behaviour, with no treatment effect ( $p = 0.91$ ). Social treatment did not affect the average litter weight at day 5 post-partum ( $p = 0.56$ ), but did affect litter weight at 4 weeks of age ( $F = 10.41$ ,  $p < 0.01$ ) and ADG ( $F = 5.97$ ,  $p = 0.01$ ). In particular, piglets from sows with intermittent daily social contact were heavier at week 4 and grew faster than those from sows housed alone, but not from sows housed in group. Piglet mortality from birth to weaning (at 4 week age) was 8.3%, with no differences between treatment groups. Eleven out of 31 sows (35.5%) had no piglet mortality. In conclusion, social contact during gestation influenced farrowing ease and postnatal piglet growth at the litter level. These findings suggest that providing social contact during gestation benefits sow welfare and piglet performance.

## Session 4

## Theatre 9

**Impact of continuous indoor housing on welfare in sheep and goats: scientific basis for grazing requirements in EU legislation**D. Dimov<sup>1</sup>, I. Nedeva<sup>2</sup><sup>1</sup> Agricultural University, 12 Mendeleev blvd., 4000 Plovdiv, Bulgaria, <sup>2</sup> Trakia University, Faculty of Agriculture, Student Town Quarter, 6000 Stara Zagora, Bulgaria

In recent decades, sheep and goat production systems in Europe have undergone increasing intensification, including the adoption of continuous indoor housing and zero-grazing systems. While such systems may improve management and productivity, their implications for animal welfare remain insufficiently addressed in current EU legislation. Sheep and goats are evolutionarily adapted herbivorous species whose biological, physiological, and behavioral needs are closely linked to grazing activity. Grazing provides not only nutritional intake but also enables locomotion, social interaction, foraging behavior, and environmental exploration, all of which are core components of animal welfare. Limiting or excluding access to grazing may therefore compromise the animals' ability to express normal behavior, as defined by internationally recognized welfare principles. This paper reviews and synthesizes scientific evidence from peer-reviewed studies and institutional reports (2010–2024) comparing continuous indoor housing with grazing-based or mixed systems for small ruminants. The analysis focuses on behavioral indicators, physiological stress responses, health outcomes, and welfare assessment frameworks. Evidence indicates that systems allowing access to grazing are associated with improved behavioral expression, reduced stress indicators, better locomotor and hoof health, and enhanced welfare compared with permanent indoor confinement. While management interventions may partially mitigate welfare limitations in indoor systems, they do not fully substitute for grazing-related behaviors. Current EU animal welfare legislation establishes welfare principles but lacks explicit requirements regarding grazing access for herbivorous livestock, in contrast to organic farming regulations. Based on the reviewed evidence, this paper argues that providing seasonal access to grazing, where environmental and climatic conditions allow should be recognized as a fundamental welfare requirement for sheep and goats. The findings support the need for a grounded revision of EU welfare standards to better align regulatory frameworks with species specific biological and behavioral needs.

**Metabolomic profiling of the heat stress response in Iberian piglets**

P. Aranguren-Rivas<sup>1</sup>, A. Heras-Molina<sup>2</sup>, E. Gómez-Izquierdo<sup>3</sup>, J. Gómez-Fernández<sup>3</sup>, F. García<sup>1</sup>, S. Bovo<sup>4</sup>, M. Bolner<sup>4</sup>, L. Fontanesi<sup>4</sup>, C. Óvilo<sup>1</sup>, J. M. García-Casco<sup>1,5</sup>, M. Muñoz<sup>1</sup>

<sup>1</sup> INIA-CSIC, Animal Breeding & Genetics Dept., 28040 Madrid, Spain, <sup>2</sup> UCM, Animal Production Dept., 28040 Madrid, Spain, <sup>3</sup> Centro de Pruebas de Porcino-ITACYL, Hontalbilla, 40353 Segovia, Spain, <sup>4</sup> University of Bologna, Dept. of Agricultural & Food Sciences, 40127 Bologna, Italy, <sup>5</sup> INIA-CSIC, R+D center in Iberian pig, 06300 Zafra, Spain

Heat stress (HS) can negatively affect swine physiology compromising its productivity. Metabolomics provides a powerful tool to uncover the biochemical mechanisms underlying these physiological changes. We investigated the impact of HS on the plasma metabolome of 20 weaned castrated male Iberian piglets (14.76 ± 2.97 kg). Animals were housed individually under thermoneutral (TN) conditions for 7 days, after which temperature was increased to 30°C for an additional 7 days. Blood samples were collected at three timepoints: before HS (T0), two days after HS onset (T2, acute), and seven days after HS onset (T7, chronic). Samples were analyzed using HPLC-based metabolomics (Metabolon Inc.). Missing values were imputed using the MICE approach, and data were corrected for fractional growth rate and batch as fixed effects, with individuals as random effects in a mixed linear model. Model residuals were used for paired timepoint comparisons using the Boruta algorithm to identify discriminant metabolites. Metabolites with a  $p < 0.05$  were considered significant using Mann-Whitney tests followed by Bonferroni correction. We identified 10 differentially abundant metabolites (DAM) between T0 and T2, 20 DAM between T0M and T7M and 36 DAM between T2 and T7, in which amino acid metabolism was the most abundant super-pathway (30.56%) and long-chain polyunsaturated fatty acids constituted the most represented sub-pathway (11.11%). The most abundant super-pathway among all the DAM was amino acid metabolism (33.70%), followed by lipid and carbohydrate metabolism (18.48% and 10.87%, respectively). These results indicate that different metabolic changes were observed between acute and chronic HS. This work has received funding from the European Union's Horizon Europe research and innovation programme under the grant agreement No. 101059609 (Re-Livestock project) and has been supported by COST Action CA22112.

## Session 4

## Theatre 11

**Early detection of disease and monitoring of welfare status using precision technologies in calves**

J. Kaler<sup>1</sup>, F. Occhuito<sup>1</sup>, M. Thomas<sup>1</sup>, J. V. Diosdado<sup>1</sup>

<sup>1</sup> University of Nottingham, School of Vet Med and Sci, College Road, LE12 5RD Sutton Bonington, United Kingdom

The developments in precision livestock technologies have enabled the collection of extensive behavioural data with high resolution for large numbers of animals. Using sensors to quantify behaviours can help identify early disease signs and develop automated detection tools. Here we used ultra-wideband location sensors and automatic milk feeders to measure a range of behaviours in dairy calves, which had not been quantified before. We derived movement behaviours including the daily distance travelled and used a novel classification and quantification algorithm to detect play behaviour. We also used the location data to determine when calves were in proximity to each other and derived social network metrics. Additionally, we used the data from the automatic feeders to extract the feeding behaviours and used an algorithm to detect displacements at the feeder. Using a variety of behaviours including movement, feeding and social network measures in 172 we were able to develop an algorithm which used the behaviours on the day to detect bovine respiratory disease with high performance (F1-score=0.7) compared to clinical health assessments carried out by researchers. This highlights the potential of using automated behavioural data to detect disease in intensive farming systems and provide timely treatment to improve animal health. Although quantifying animal welfare is challenging, play behaviour is often seen as a measure of positive welfare. In calves we were able to detect play with an algorithm that achieved great performance (F1-score=0.9) compared to visual observations and showed that play decreased significantly with disease ( $\beta = -0.27$ ,  $SE = 0.02$ ,  $p < 0.01$ ,  $N = 226$ ) and increased when calves were provided with enrichment ( $\beta = 0.43$ ,  $SE = 0.11$ ,  $p < 0.01$ ,  $N = 226$ ). Overall, these results highlight that using precision technologies allows for the detection of a wide range of behaviours, with the potential to create an automated system for the monitoring of farm animal health and welfare. This type of monitoring, which can be applied to large numbers of animals and for extended periods of time, is essential to develop a better understanding of the effects of management interventions on farm animals.

**Mass mortality of adult dairy cows associated with *Clostridium perfringens* alpha toxin.**K. Rypuła<sup>1</sup>, K. Płoneczka-Janeczko<sup>1</sup>, A. Zielak-Steciwko<sup>2</sup>, M. Siedlecka<sup>1</sup><sup>1</sup> Wrocław University of Environmental and Life Sciences, Department of Epizootiology with Clinic for Birds and Exotic Animals, Plac Grunwaldzki 45, 50-366 Wrocław, Poland, <sup>2</sup> Wrocław University of Environmental and Life Sciences, Institute of Animal Breeding, Chelmońskiego 38C, 51-631 Wrocław, Poland

*Clostridium perfringens* infections in adult cattle are rare, and only a few clinical cases of type A associated with toxemia in this age group have been reported. The aim of this study is to describe an unusual mass occurrence of disease and mortality in adult dairy cattle in Poland. The event concerned 30 animals experiencing unexpected fatalities among high-yielding periparturient cows. Nutritional records showed the provision of a TMR that included a new part of straw. Clinical symptoms included a sudden decrease in milk production, movement difficulties, vomiting, rapid death, and the leakage of liquid blood from natural body orifices with impaired clotting. A post-mortem examination, biochemical serum analysis, microbiological testing of organs/milk (culture, MALDI -TOF, PCR), and toxicological testing were conducted (n=4) to identify the cause of death. Post-mortem examination revealed widespread bleeding lesions, such as hemorrhagic enteritis, petechiae on internal organs, hepatic congestion, bloody effusions in body cavities, and pulmonary emphysema. The tested serum parameters were within reference ranges. Toxicological assessments ruled out the participation of mycotoxins and the possibility of poisoning from plant protection products. PCR identified the presence of alpha toxin gene of *Clostridium perfringens* in the kidneys. Mass deaths occurred due to the synergistic effects of diet alterations, environmental influences, and decreased immunity. The shift to a diet rich in carbohydrates and proteins encouraged anaerobic gut conditions that favored the growth of *Clostridium perfringens*, whereas inadequately dried straw may have aided spore survival. Immunosuppression linked to the periparturient period increased the animals' susceptibility to illness. Toxin production caused damage to the endothelium, hemolysis, and tissue necrosis, leading to death. Consideration should be given to implementing immunoprophylaxis against *C. perfringens* type A and monitoring toxigenic strains.

## Session 4

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**Overcoming thermosensitivity in mare's milk: A synergistic sterilization protocol validated by impedance monitoring**M. S. Lara<sup>1</sup>, M. I. Berruga<sup>1</sup>, A. M. Molina<sup>1</sup><sup>1</sup> University of Castilla-La Mancha, Agri-Food and Industrial Biotechnology Group, Av. de España, s/n, 02001 Albacete, Spain

**Abstract**— The aim of this work was to develop and validate a sterilization protocol for thermosensitive mare's milk capable of ensuring microbiological safety without destabilizing its albuminous matrix. A secondary objective was to compare the accuracy of traditional counting techniques against high-sensitivity impedance systems for detecting metabolic activity. A hurdle technology experimental design was applied, screening over 30 combinations of thermal stress, microwaves, and UV-C radiation. Microbiological validation employed a dual-verification strategy: classic methods using Plate Count Agar (PCA) and Reinforced Clostridial Agar (RCA), versus advanced real-time monitoring using the BacTrac impedance system. This allowed for the detection of metabolic kinetics and bacterial recovery often missed by standard plating. Comparative analysis revealed significant discrepancies, where the BacTrac system exposed bacterial survival in treatments that appeared effective on agar plates (false negatives). While isolated thermal treatments caused protein destabilization, the impedance curves allowed the identification of a unique synergistic combination of thermal and non-thermal barriers. This specific protocol achieved total metabolic inhibition while maintaining the physical integrity of the milk. This study establishes a valid alternative for mare's milk commercial sterility, superior to conventional thermal processing. The integration of hurdle technologies, validated by high-precision impedance microbiology, offers a robust solution for the industrialization of this functional dairy product. **Keywords**— Mare's milk, Hurdle technology, Impedance microbiology, Microwaves

**Impact of fattening season on the gut microbiome of free-range Iberian pigs**

J. M. García Casco<sup>1,2</sup>, M. Muñoz<sup>2</sup>, A. López García<sup>2</sup>, Á. González Berdasco<sup>2</sup>, R. Peiró<sup>2</sup>, P. Palma Granados<sup>1,2</sup>

<sup>1</sup> INIA-CSIC, R&D Center for Iberian Pig, Ctra Ex 101 km 4.7, 06300 Zafra, Spain, <sup>2</sup> INIA-CSIC, Animal Breeding, Ctra La Coruña km 7.5, 28040 Madrid, Spain

In Iberian pig production, cebo de campo refers to free-range fattening without access to acorns consumed in the autumn–winter dehesa. These pigs are raised year-round, with  $\geq 100$  m<sup>2</sup> per animal, a minimum of 60 days outdoors before slaughter, and a diet based on commercial feeds plus seasonal natural resources when available. Due to the climate of southwestern Spain (mild winters, temperate rainy springs and autumns, and increasingly long, hot, dry summers), outdoor-fattened pigs face highly variable conditions that affect resource availability, land-use behaviour, welfare, and potentially their metabolism and microbiome. Therefore, this study aimed to analyse the intestinal microbiome composition of four free-range fattening batches across seasons to identify differences with potential implications on production traits, animal welfare and health. Faecal samples of 120 Iberian pigs from four batches (n = 30) were collected by swabbing on the days preceding to the slaughter: Dec-21, Jul-11, Aug-22, and Apr-3; therefore, the fattening periods corresponded to autumn, spring, summer, and winter. Beta-diversity analyses revealed clear differences in microbial composition between pigs slaughtered in Apr&Dic and those slaughtered in Jul&Aug, when the climatic conditions were very hot and there was no vegetation cover available. The differentially abundant analyses showed 106 genera that were significantly different between both groups. Genera more abundant in Apr&Dec, such as *Streptococcus*, are associated with rapid fermentation, marked by increased lactate and propionate production, typical of higher simple carbohydrate intake or lower fibre consumption. In contrast, genera more abundant in Jul&Aug, including members of the *Lachnospiraceae* family, are linked to fibre fermentation, characterised by butyrate production, enhanced polysaccharide degradation, and increased anabolic pathways, reflecting diets richer in plant material and complex fibres. These results highlight seasonal differences in the microbiota of fattened animals and will be complemented by studies on productivity, welfare, and differential gene expression.

## Session 4

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**Effects of housing types and seasonal variations on the performance and welfare of Uda rams**

K. M. Aljameel<sup>1</sup>, S. A. Maigand<sup>2</sup>

<sup>1</sup> Usmanu Danfodiyo University, Sokoto, Animal Science, Wammako, 234 Sokoto, Nigeria, <sup>2</sup> Usmanu Danfodiyo University Sokoto, Animal Science, Wammako, 234 Sokoto, Nigeria

This study was conducted at Department of Animal Teaching and Research Farm (UDUS) to evaluate the effects of housing types and seasonal variations on the performance and welfare of Uda rams. A factorial design (3×5) was used in this experiment with number of animals representing replicates while housing types and season representing the factors (treatment combination). Four animals were allotted to each housing type with each animal serving as replicate. Twenty yearling rams were allocated to five housing types full wall with zinc roofing (FZ), full wall with thatch roofing (FT), half wall with zinc roofing (HZ), half wall with thatch roofing (HT), and no wall/shade (N) and studied across hot, wet, and cold seasons. Performance parameters, feed intake, stress indicators (rectal temperature, respiratory rate, pulse rate) and thermal comfort indices (THI, BGHI, TCI). Data were subjected to correlation and regression analysis. Results indicated that environmental stress negatively affected feed intake and weight gain. Fully walled and shaded housing reduced physiological stress, improved thermal comfort, and enhanced growth and reproductive performance, whereas rams housed without walls or shade exhibited elevated stress and poorer performance. There result showed negative correlation between weight gain and feed intake with Dbt, Wbt, relative humidity, TCI, BGHI and THI. FCR was negatively correlated with dry bulb temperature (Dbt), thermal comfort index (TCI) and temperature humidity index (THI). These findings highlight the critical role of housing and seasonal management in mitigating heat stress and optimizing growth and sustainability of Uda rams.

### The impact of age at first mating on lifetime productivity in Alpine goats

A. Kasap<sup>1</sup>, D. Mulč<sup>2</sup>, B. Mioč<sup>1</sup>, V. Držaić<sup>1</sup>, Z. Prpić<sup>1</sup>, D. Jurković<sup>3</sup>, Z. Barać<sup>4</sup>, M. Špehar<sup>3</sup>

<sup>1</sup> University of Zagreb Faculty of Agriculture, Svetošimunska 25, 1000 Zagreb, Croatia, <sup>2</sup> Ministry of Agriculture, Ulica grada Vukovara 78, 1000 Zagreb, Croatia, <sup>3</sup> Croatian Agency for Agriculture and Food, Svetošimunska 25, 10000 Zagreb, Croatia, <sup>4</sup> Croatian Association of sheep and goat breeders, Jarnovičeva 17c, 10000 Zagreb, Croatia

**Summary** This study investigated the relationship between age at first mating (AFM) and milk yield across the productive lifespan of Alpine goats in Croatia, utilizing data collected in accordance with ICAR standards provided by the Croatian Agency for Food and Agriculture. The analysis was based on a dataset comprising 3,200 lactation records from 720 individual goats across three commercial herds. To determine the effect of AFM on milk yield, a linear mixed-effects model was employed, accounting for the fixed effects of AFM and its interaction with parity, as well as litter size, goat's age, suckling and milking durations, season, and herd, while incorporating the individual goat as a random effect to control for repeated measures. Additionally, the impact of AFM on lifetime production (defined by the total number of lactations and cumulative milk yield) was analysed using a regression model restricted to culled animals. The results reveal a significant trade-off between early-life productivity and long-term biological efficiency. While initial regression analysis showed that goats with a lower AFM produce less milk in their first and second lactations (15.20 kg and 6.47 kg less per month of age, respectively), this deficit is effectively offset by an extended productive lifespan. Specifically, for every month of delay in first mating, goats lose 0.1027 of a lactation, leading to a significant reduction of 34.11 kg in total lifetime milk yield per one month of delay of first mating. These findings confirm that the higher initial yields observed in goats mated at an older age are insufficient to compensate for the cumulative loss caused by their shortened productive life. Ultimately, mating goats at an earlier age (while not below 7 months) leads to superior lifetime milk yield and improved economic sustainability for Alpine goat breeders. **Key words:** Alpine goat, age, first mating, parity, milk yield

## Session 4

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### Practical aspects of extensive sheep grazing in Poland, with particular emphasis on mountainous areas

M. Puchala<sup>1</sup>, R. Garstka<sup>1</sup>, A. Kawecka<sup>1</sup>, M. Pasternak-Chorosz<sup>1</sup>, I. Peist<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, Department of Sheep and Goat Breeding, 2 Sarego, 31-047 Krakow, Poland

The main method of sheep management in Poland is the pasture-and-shed system. In mountainous areas, a purely grazing system is common. Mountainous areas under various forms of nature conservation are predisposed to extensive animal production. In addition to providing conditions for the development of the market for regional and traditional foods closely related to native sheep breeds, they also constitute an effective tool for landscape conservation. A project commissioned by Ministry of Agriculture and Rural Development analysed the use of sheep grazing in various regions of the country. The analysis took into account many aspects of breeding as a: the selection of grazing technology to meet the contemporary requirements of nature conservation and animal welfare, the assessment of grazing as an element of environmental programmes. Breeders indicated that, given low profitability of sheep farming, utilizing grazing is crucial as the cheapest and most effective source of feed. This approach reduces costs, labour input and time, and promotes the preservation of grassland biodiversity. Farmers also emphasized possibility of utilizing land unsuitable for any other purpose. In most regions of Poland, sheep farming focuses on the production of slaughter lambs, which is one of the main sources of income for farms derived from livestock. Lambs, once they reach a weight of 40-45 kg, are mainly sold for export, due to unstable domestic demand. However, the use of sheep for dairy purposes in Poland is limited primarily in mountainous regions, where milk processing plays a significant role. Other products derived from sheep farming, particularly wool and hides, are perceived by many farmers as unprofitable. Most respondents reported difficulties in managing and selling them, citing low purchase prices, often below the break-even point, despite growing consumer interest in regional and traditional products. Other problems among the surveyed farms included rising labour costs, a decreasing number of young people interested in taking over the farm, losses caused by predators attacking grazing flocks, and the high prices of medications, veterinary services, shearing, and fertilizers.

### Effect of heat stress in lactating Sarda ewes kept in a barn with a roof ventilator

M. A. Porcu<sup>1</sup>, F. Castangia<sup>1</sup>, A. Ledda<sup>1</sup>, A. Cannas<sup>1</sup>

<sup>1</sup> Department of Agricultural Sciences, Viale Italia 39, 07100 Sassari, Italy

Heat stress (HS) affects physiological and productive responses of ewes according to breed and physiological stage. However, ventilation systems can modify the ewe's physiological and productive responses by promoting heat dissipation. Therefore, this study investigated the responses of 43 lactating dairy sheep housed indoors with a roof ventilation system to HS during two trials. Rectal temperature (RT; °C) and respiration rate (RR; breaths/min) were measured on three experimental days (23 May, 6 and 13 June 2025) during Trial 2, and only on the last two dates during Trial 1. Measurements were performed three times per day at 5-hour intervals (06:00, 11:00, and 16:00 h). Temperature-Humidity Index (THI) was calculated for each hourly recording during the experimental days using the equation of Kliber (1964). Data were analyzed using ANOVA, and relationships were assessed using linear regression analysis. The average daily THI increased from the first to the third experimental day (69 and 79, respectively;  $P < 0.001$ ) and from 06:00 to 16:00 h on all experimental day ( $P < 0.001$ ). The RR was significantly affected by the day ( $P < 0.001$ ) and hour ( $P < 0.001$ ). The RR increased from the 1st to the 3rd experimental day (mean values: 51 and 88 breaths/min, respectively), and from 0600 h to the 1600 h in each experimental day ( $P < 0.001$ ). A significant positive relationship was found between RR and THI at the time of recording ( $y = 3.58x - 200.67$ ;  $R^2 = 75$ ;  $P < 0.001$ ); the relationship was weaker considering the average daily THI ( $R^2 = 0.38$ ;  $P < 0.001$ ). Additionally, the RR was strongly and positively correlated with air temperature at the time of recording ( $y = 5.49x - 70.77$ ;  $R^2 = 0.78$ ;  $P < 0.001$ ). The RT increased from 38.8 °C at THI  $< 70$  to 39.5 °C at THI  $> 80$  ( $P = 0.03$ ). A positive relationship was found between RR and RT ( $R^2 = 0.31$ ,  $P < 0.001$ ). The total DMI, milk yield and energy balance were not affected by the THI. In conclusion, THI significantly affected the RR and RT, particularly the THI at the time of recording. However, in this trial it did not affect ewe DMI and productivity, probably likely due to the presence of a ventilation system that limited the ewe's physiological responses to HS, representing a useful mitigation tool against negative effects of thermal stress.

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### Colostrum quality in different goat's breed

N. M. Palladini<sup>1</sup>, L. Bava<sup>1</sup>, A. Sandrucci<sup>1</sup>, A. Tamburini<sup>1</sup>, G. Gislone<sup>1</sup>, P. Baronchelli<sup>1</sup>, S. Bonizzi<sup>1</sup>, M. Pavolini<sup>1</sup>, S. Sari<sup>1</sup>, M. Zucali<sup>1</sup>

<sup>1</sup> University of Milan, Agricultural and Environmental science, Via Celoria 2, 20133 Milan, Italy

Eight dairy goat farms, located in Northern Italy, were selected, representing four different breeds: Saanen, Toggenburg, Camosciata, and Nubiana. The farms had on average 56 ( $\pm 21$ ) lactating goats. 14 ( $\pm 1.4$ ) samples per farm were obtained, for a total of 59 Camosciata, 15 Nubiana, 23 Saanen, and 15 Toggenburg samples. Analyses were performed to determine colostrum immunological quality, in terms of °Brix (refractometer) and  $\gamma$ -globulins concentration (electrophoresis). In addition, a questionnaire was administered to the farmers for evaluating colostrum management practices, focusing on administration and storage methods. 67% of the farms used a colostrum bank. All farmers administered colostrum ad libitum, using a baby bottle within 6 hours after birth; however, only 33% of the farms performed colostrum analysis (using refractometer) to assess quality. Average colostrum quality was 22.6 ( $\pm 6.2$ ) °Brix and 41.9 ( $\pm 20.7$  g/L) of  $\gamma$ -globulins. Variability among the farms was high and, on average, the minimum colostrum quality threshold was not reached. Only four farms, in fact, achieved a  $\gamma$ -globulins concentration of 50 g/L, although six farms recorded colostrum values above 22 °Brix. Regarding  $\gamma$ -globulins concentration, Nubiana colostrum was higher than that of Camosciata and Saanen ( $p < 0.05$ ), while Toggenburg and Camosciata were higher than Saanen ( $p < 0.05$ ). In terms of °Brix, Saanen colostrum showed significantly lower quality. This study showed that variability in colostrum management among farms is high, with some farms adopting recommended protocols. In fact, 50% of the farms did not reach the minimum  $\gamma$ -globulins concentration in colostrum. The differences observed among breeds are noteworthy and are likely related to genetic selection and specialization in milk production; the higher quality of colostrum produced by some local breeds may represent an adaptive characteristic that helps the growth and survival of the kids, as reported by literature. These findings are particularly relevant for Mediterranean goat production systems, where the preservation and valorisation of local breeds play a key role in resilience and sustainability.

**Drinking water microbiology and mortality in beef calves under extensive conditions**A. Benito Díaz<sup>1</sup>, M. Montañés Foz<sup>1</sup>, R. Bodas Rodríguez<sup>1</sup>, A. Sarmiento García<sup>2</sup>, J. J. García García<sup>1</sup><sup>1</sup> *Technological Agricultural Institute of Castile and León (ITACyL), Ruminant Research Unit, Burgos-Portugal road, km 119, 47071 Valladolid, Spain,* <sup>2</sup> *University of León, Department of Animal Production, Vegazana Campus, s/n, Faculty of Veterinary Medicine, 24007 León, Spain*

Drinking water quality is frequently considered a relevant component of health management in extensive beef cattle systems, where animals may have access to non-chlorinated water sources. This study evaluated the association between the microbiological quality of drinking water and calf mortality across different age stages in beef cattle farms located within the same region of Castile and León (Spain). A total of 22 drinking water samples from 22 farms were analysed for aerobic colony counts at 22 °C, coliforms, *Escherichia coli*, enterococci and *Clostridium perfringens*. In parallel, mortality data were recorded at the farm and classified into three age categories: 0–2 days, 3–15 days and >15–365 days. Associations between microbiological parameters and mortality were assessed using analysis of variance. No statistically significant relationships were observed between any of the analysed microbiological variables and mortality in any age category ( $p > 0.05$ ). No evidence of association was detected for neonatal mortality (0–2 days) or for deaths occurring between 3 and 15 days of age. A trend ( $p < 0.10$ ) towards higher mortality in calves older than 15 days was observed in farms where *C. perfringens* was detected in drinking water. Despite the geographical proximity of the some farms, substantial variability in microbiological water quality was observed between holdings. Overall, these findings suggest that drinking water microbiology alone has limited explanatory value for calf mortality in extensive beef systems. The results support a multifactorial interpretation of losses and indicate that the role of *Clostridium perfringens* should be further explored in larger studies.

**Grape stem inclusion in the diet of fattening young bulls affected erythropoiesis in the short-term**I. Casasús<sup>1</sup>, D. Villalba<sup>2</sup>, S. Lobón<sup>1</sup>, M. Joy<sup>1</sup>, C. Conesa<sup>1</sup>, M. Blanco<sup>1</sup><sup>1</sup> *CITA - IA2, Av Montañana 930, 50059 Zaragoza, Spain,* <sup>2</sup> *Universitat de Lleida, Av Rovira Roure 191, 25198 Lleida, Spain*

To promote circularity, grape (*Vitis vinifera*) stems, a fibrous agro industrial by product, can be included in beef fattening diets at low doses (up to 6%) without deleterious effects on performance. The polyphenols present in grape have antioxidant and anti-inflammatory effects, but their iron-chelating properties may impair erythropoiesis. However, most studies examined grape pomace or other byproducts rather than grape stems, with potentially different polyphenolic profiles. Young bulls (162 days old; 173 kg BW,  $n=24$ ) were fed iso-energetic (1.04 FU/kg DM) and iso-proteic (11.2% DM) concentrates ad libitum for 17–20 weeks. Concentrates contained different proportions of grape stem and barley straw in inverse ratios: 0% stem (6% straw), 3% stem (3% straw) and 6% stem (0% straw). Bulls were bled during the pre-experimental period, after 3 weeks and 19 weeks on concentrates. A complete blood count was performed, including variables related to the erythrocyte (red blood cell) series, leukocyte (white blood cell) series, and platelet parameters. The antioxidant status was determined through malondialdehyde (MDA) concentrations. Regarding the red blood cell parameters, haematocrit and haemoglobin were greater in 6%stem than in 0% stem after 3 weeks on the diets, but differences disappeared after 19 weeks. Mean corpuscular volume was greater in 6%stem than in 0%stem only after 19 weeks. In the white series, total leukocytes were not affected by the proportion of grape stems, but the granulocyte populations differed after 19 weeks. The percentage of neutrophils was greater in 3% than in 6%stem bulls whereas the percentage of lymphocytes tended to be lower in 3%stem bulls than in their counterparts. Consequently, neutrophil:lymphocyte ratio was greater in 3%stem bulls than 6%stem bulls. Neither platelet counts nor MDA concentrations were affected by the proportion of grape stem. In conclusion, our results do not support a negative effect of grape stem on erythropoiesis, and rather indicate a positive initial response. However, we could not confirm an anti-inflammatory effect of grape stem or a reduction of oxidative stress of young bulls.

**Assessment of Seasonal Variability in Milk Production and Hygienic Quality in a Farm Applying a Pasture–Indoor System***I. Radkowska<sup>1</sup>, A. Radkowski<sup>2</sup>*<sup>1</sup> *National Research Institute of Animal Production, Department of Cattle Breeding, Sarego 2, 31-047 Krakow, Poland,*<sup>2</sup> *University of Agriculture, Department of Agroecology and Plant Production, Mickiewiczza 21, 31-120 Krakow, Poland*

The aim of the study was to evaluate seasonal changes in milk yield, chemical composition, and hygienic quality parameters of milk in a dairy farm applying a mixed management system, including pasture-based feeding during the spring–summer period and indoor housing during the autumn–winter period. The analysis covered monthly milk production over a full annual cycle (I–XII), fat and protein content, as well as indicators of hygienic milk quality, namely somatic cell count (SCC) and total bacterial count (TBC). During the winter period (I–IV), the average milk yield amounted to 27,525 liters, while maintaining high and stable hygienic quality of the raw milk. Low SCC and TBC values were recorded, indicating good udder health and effective hygiene control under indoor housing conditions. These results are consistent with literature reports demonstrating more favorable hygienic parameters of milk during the winter season. With the onset of the grazing period (V–IX), a significant increase in milk yield was observed, averaging 18%. This phenomenon was primarily associated with the seasonality of calvings and a higher proportion of cows in the early and mid-stages of lactation. At the same time, a deterioration in the hygienic quality of milk was noted, expressed by an increase in SCC and a periodic rise in TBC. These changes may have been caused by high ambient temperatures and the occurrence of heat stress. After the end of the grazing season (X–XII), an improvement in the hygienic quality parameters of milk and stabilization of its chemical composition were observed. In conclusion, the pasture–indoor system applied in the analyzed farm promoted higher milk yield during the summer period; however, it requires intensified measures related to milking hygiene and mastitis prevention in order to maintain high technological quality and microbiological safety of milk. Funding: Project Network of Universities of Life Sciences—Development of Innovative Dairy Farming (SUP-RIM) financed from the funds of the Ministry of Education and Science based on agreement no MEiN/2023/DPI/2872.

**Research Projects and International Cooperation of the National Research Institute of Animal Production in the Field of Animal Health and Welfare under Climate Change Conditions***A. Orchel-Szeląg<sup>1</sup>*<sup>1</sup> *National Research Institute of Animal Production, 2 Sarego St., 31047 Kraków, Poland*

Animal health and welfare are key elements of sustainable livestock production, influencing farm efficiency. In recent years, the National Research Institute of Animal Production (NRIAP) has intensified research activities focused on improving animal welfare and health, as well as adapting livestock systems to climate change. Analysis of project activity over the last five years shows financial engagement from national funds and European Union programmes. Within governmental research grants, the Institute annually carries out research and development tasks worth from several to over twenty million PLN. These projects cover animal health and welfare assessment, housing system improvements, and analysis of environmental impacts on livestock production. National funding is complemented by EU programmes such as Horizon Europe and the European Partnership on Animal Health and Welfare (EUP AHW), strengthening research capacity and cooperation. An important area of activity is participation in projects under the Rural Development Programme (EIP-AGRI), enabling cooperation between science and agricultural practice. These projects, with budgets reaching several million PLN, focus on implementing innovative on-farm solutions improving animal welfare, reducing production losses, and supporting adaptation to climate change. Due to increasing heat waves and extreme weather events, research on heat stress in livestock is crucial. Chronic heat stress causes behavioural and physiological changes, leading to long-term welfare deterioration. NRIAP projects address these challenges by improving microclimate conditions in barns, milking parlours, and calf housing, which helps reduce heat stress, improve animal health and behaviour, and enhance working conditions for farm staff, while also supporting farmers' well-being by reducing workload pressure, economic losses, and occupational stress. Integration of scientific research with practical farm implementation, combined with broad national and international cooperation, supports the development of effective solutions improving animal welfare, farmers' well-being, and the sustainability of livestock production under climate change conditions.

**Welfare of Farm Animals Used in Scientific Research in Research Institutes in Poland**A. Orchel-Szelqg<sup>1</sup><sup>1</sup> National Research Institute of Animal Production, 2 Sarego St., 31047 Kraków, Poland

The welfare of farm animals used in scientific research is an important element of responsible research conduct and a prerequisite for obtaining reliable scientific results. In Poland, research institutions conducting animal studies operate in accordance with national legislation and Directive 2010/63/EU on the protection of animals used for scientific purposes, ensuring high ethical and husbandry standards. Statistical data indicate that the number of animals used in research in Poland is moderate compared with the EU average. In recent years, approximately 120,000–250,000 procedures involving animals have been recorded annually. Farm animals constitute a small share of the total number of animals used – for example, cattle account for about 0.2% and pigs about 0.5%. Despite this low numerical share, research involving farm animals has high economic and social importance, particularly in relation to dairy and beef cattle. Research involving cattle mainly focuses on health, nutrition, reproduction, welfare, and adaptation to changing environmental conditions, including heat stress. Particular importance is placed on the evaluation of housing conditions, barn microclimate, dairy cattle housing systems, and the impact of environmental factors on productivity and animal health. Increasing attention is also given to monitoring welfare indicators such as behaviour, physiological parameters, health status, and stress levels. Research institutes are continuously developing infrastructure to ensure appropriate animal housing conditions, including proper microclimate, sufficient living space, and environmental enrichment. An important aspect is the implementation of the 3R principle (Replacement, Reduction, Refinement), aimed at reducing animal numbers and minimizing discomfort during research. The integration of scientific research, ethical supervision, and national and international cooperation supports the development of modern standards for keeping farm animals used in research, contributing to scientific progress while improving animal welfare protection.

## Session 4

## Poster 25

**Effect of Lemon Balm (*Melissa officinalis*) Supplementation on Stress-Related Behavior and Welfare in Weaned Pigs**S. Ivanova<sup>1</sup>, M. Petrova<sup>1</sup><sup>1</sup> Agricultural Academy, Agricultural Institute, 3 Simeon veliki bulvd., 9703 Shumen, Bulgaria

The aim of the experiment was to determine the effect of supplementing the diet with dried leaf mass of lemon balm (*Melissa officinalis*) on positive behavior and selected blood parameters in growing pigs. A scientific experiment was conducted at the Agricultural Institute – Shumen, involving a total of 48 growing pigs of the Danube White breed, weaned at age of 40 days, with an average live weight of 9.233 kg. The pigs were equalized by origin, age, live weight, and sex. There are two treatments: a control group (CON), and an experimental treatment (MELOF). Pigs from MELOF received gradually from 1 to 7 g per pig per day lemon balm (*Melissa officinalis*) for 55 days. Piglet behavior in both treatments was continuously monitored by video surveillance 24 hours a day over two consecutive days. Behavioral data were collected from 08:00 to 18:00 on the 1st and 2nd day after weaning, two days before the end of the experimental period, as well as immediately after the transfer of pigs to the fattening unit. A scan sampling method was applied at 2-minute intervals by a single trained observer. At the present stage, only active and inactive behaviors from the ethogram have been processed. Based on 600 observations at two-minute intervals for each group at the beginning and at the end of the experimental period, it was established that supplementation with dried lemon balm leaf mass in the diet of MELOF, had a significant effect ( $P < 0.001$ ) on behavioral activity at the end of the experiment. This result is most likely due to the calming effect of lemon balm. Regarding the hematological results, weaned pigs from MELOF treatment showed a statistically significant increase in WBC ( $P = 0.048$ ), LYM ( $P = 0.008$ ), and MON ( $P = 0.004$ ) compared to the CON, while the proportion of granulocytes (GRA%) was significantly lower ( $P = 0.048$ ). At this stage of the study, the results indicate a potential of an immunomodulatory and anti-stress effect of lemon balm. Future analyses of serum serotonin and cortisol concentrations are envisaged to elucidate the neuroprotective potential of lemon balm. This project is implemented with the support of COST Action CA21124 LIFT and a contract with the Bulgarian Science Fund, No. KP-06-COST/20 from 12.08.2024.

### Hoof surface temperature assessed by infrared thermography in dairy cattle in relation to breed and lactation

W. Zajac<sup>1</sup>, M. Soroko-Dubrovina<sup>1</sup>, E. Pecka-Kielb<sup>2</sup>, A. Zachwieja<sup>1</sup>, M. Wnętrzak<sup>3</sup>, A. Zielak-Steciwko<sup>1</sup>

<sup>1</sup> Wrocław University of Environmental and Life Sciences, Institute of Animal Breeding, Chelmońskiego 38, 51-631 Wrocław, Poland, <sup>2</sup> Wrocław University of Environmental and Life Sciences, Department of Biostructure and Animal Physiology, Norwida 31, 50-375 Wrocław, Poland, <sup>3</sup> Wrocław University of Environmental and Life Sciences, Department of Mathematics, Grunwaldzka 53, 50-357 Wrocław, Poland

Lameness in dairy cattle is a major health problem, leading to reduced milk yield and significant economic losses. This study aimed to evaluate the effects of breed, lactation number, and days in milk (DIM) on hoof surface temperature in clinically healthy dairy cows, in order to support the potential use of infrared thermography for early lameness detection. All animals originated from a single herd and were managed under identical housing and feeding conditions. Thermal images of the dorsal surfaces of the hind hooves were obtained, using a HIKMICRO M30 infrared camera, from 132 lactating cows representing three breeds: Polish Holstein–Friesian (HF, n=49), Red-and-White (ZR, n=41), and Polish Red (RP, n=42). Data on lactation number and DIM were recorded for each animal. The highest hoof surface temperatures were observed in HF cows, whereas the lowest values were recorded in ZR cows. RP cows exhibited the narrowest temperature range, indicating lower within-population variability. However, inter-breed differences were not statistically significant ( $p>0.05$ ). Hoof surface temperature tended to be slightly higher in cows in the 3rd and subsequent lactations compared with those in the 1st and 2nd lactations and remained relatively stable throughout lactation, although cows with DIM  $>300$  showed marginally higher values. Neither parity nor DIM had a significant effect on hind hoof surface temperature ( $p>0.05$ ). In conclusion, in clinically healthy dairy cows, breed, lactation number, and DIM do not significantly influence hoof surface temperature. These findings support the use of thermography as a universal physiological-independent diagnostic tool. The research is co-financed under the individual student research project „Młode umysły – Young Minds Project” from the subsidy increased for the period 2020–2026.

## Session 4

## Poster 27

### Effects of a low crude protein diet with reduced amylose-to-amylopectin ratio on performance, welfare, gut microbiota and meat quality in heavy pigs

P. Trevisi<sup>1</sup>, M. V. Graziosi<sup>1</sup>, F. Soglia<sup>1</sup>, M. Petracci<sup>1</sup>, F. Correa<sup>1</sup>, D. Luise<sup>1</sup>

<sup>1</sup> University of Bologna, Department of Agricultural and Food Sciences, Viale G. Fanin 46, 40127 Bologna, Italy

This study investigated the effects of a low-CP diet combined with a reduced amylose-to-amylopectin ratio (AM:AP) on performance, behaviour, faecal microbiota, carcass traits, and fatty acid (FA) composition in growing–finishing pigs. A total of 384 pigs (initial body weight 36.8 kg) were allocated to a control diet (CO; AM:AP=17) or a treated diet (TRT; CP reduced by 1.5%, 1.4% and 0.7% across three feeding phases; AM:AP=7), balanced for sex and body weight. The reduced AM:AP in TRT was achieved by replacing conventional maize with waxy maize. Growth performance and behavioural indices were monitored throughout the trial. Faecal samples (16 pigs/group) were collected at d0, d36 and d160 to assess microbiota composition (16S rRNA gene sequencing). At slaughter ( $\approx 160$  days), carcass traits (20 pigs/group) were evaluated. Dietary treatment did not affect growth performance. TRT pigs showed greater activity at d50 ( $P=0.03$ ), with no differences in lesions. Faecal microbiota was affected by a diet $\times$ time interaction ( $P<0.0001$ ), with higher abundance of *Clostridium sensu stricto 1* and *Terrisporobacter* in TRT pigs at d36 and d160 ( $P\leq 0.03$ ). Carcass weight and the proportion of carcasses suitable for Parma PDO ham production were unaffected by diet. The TRT diet tended to increase fat thickness and reduce muscle thickness ( $P\leq 0.10$ ). Drip loss was higher in the shoulder of TRT pigs ( $P=0.01$ ), while semimembranosus muscle showed higher drip loss and cooking loss ( $P=0.03$  and  $P=0.04$ ). In biceps femoris, pH at 24 h post mortem was higher in TRT pigs ( $P=0.05$ ). The TRT diet modulated FA composition in a tissue-specific manner, reducing monounsaturated FA in shoulder ( $P=0.05$ ), while increasing monounsaturated and reducing polyunsaturated,  $\omega 6$  and  $\omega 3$  FA in biceps femoris ( $P\leq 0.02$ ). Backfat FA composition was minimally affected. In conclusion, a low-CP diet with a reduced AM:AP ratio maintains growth performance, carcass suitability for PDO production, and overall meat quality, supporting its potential as a sustainable feeding strategy for heavy pig production. Acknowledgements The authors would like to acknowledge Dr. Luca Campidonio

**Identification of mutations in selected exons of the CLPTM1 gene responsible for cleft palate in the domestic dog (*Canis lupus familiaris*) in the French Bulldog breed**

*W. Jabłońska*<sup>1,3</sup>, *K. Węglińska*<sup>1</sup>, *A. Sójka*<sup>1</sup>, *B. Grzegorzółka*<sup>1,2</sup>, *J. Gruszczyńska*<sup>2</sup>

<sup>1</sup> *Warsaw University of Life Sciences – SGGW, Scientific Association of Experimental and Laboratory Animals, Ciszewskiego 8, 02-786 Warsaw, Poland,* <sup>2</sup> *Warsaw University of Life Sciences – SGGW, Department of Animal Genetic and Conservation, Ciszewskiego 8, 02-786 Warsaw, Poland,* <sup>3</sup> *University of Silesia in Katowice, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Science, Jagiellońska 28, 40-032 Katowice, Poland*

Cleft lip and/or palate (CL/P) is a congenital defect in the domestic dog (*Canis lupus familiaris*) involving abnormal fusion of palatal tissues during embryogenesis. The CLPTM1 gene in domestic dog is a homolog of human CLPTM1 gene. The aim of this study was to identify mutations in 14 exons of the CLPTM1 gene determining the incidence of CL/P in the dogs (French Bulldog). The material was tissues of 5 individuals (mother without CP, offspring with CP). DNA was isolated by column method and purified. Exons of CLPTM1 gene were amplified by PCR reaction and sequenced. Bioinformatics analysis of the sequences was performed by Chromas 2.6.6 and BioEdit 7.7.1 software, and obtained nucleotide sequences were compared with gene's reference sequence. No mutations were detected in the nucleotide sequences in 10 of 14 exons. In exons 2, 3 and 13 in 4 individuals (60 – exon 2; 60, 61, 62 – exon 3; 63 – exon 13), transversions, transpositions, insertions were identified as synonymous mutations. In exon 1 and 14A, variation could't be estimated. Results suggest that CL/P may be caused by mutations in CLPTM1 gene. Further studies on the function and non-regulatory sites of CLPTM1 gene are needed.

**Congenital bone fragility in the domestic dog is determined by mutations within the genes: COL1A1, COL1A2, SERPINH1**

*D. Goworek*<sup>1</sup>, *J. Owczarek*<sup>1</sup>, *B. Grzegorzółka*<sup>1,2</sup>, *J. Gruszczyńska*<sup>2</sup>

<sup>1</sup> *Warsaw University of Life Sciences – SGGW, Scientific Association of Experimental and Laboratory Animals, Ciszewskiego 8, 02-786 Warsaw, Poland,* <sup>2</sup> *Warsaw University of Life Sciences – SGGW, Department of Animal Genetic and Conservation, Ciszewskiego 8, 02-786 Warsaw, Poland*

The COL1A1 gene is located on chromosome 9 in the domestic dog and is the precursor for a single unit of collagen. Mutations within the COL1A1, COL1A2 genes are inherited in an autosomal dominant manner and within the SERPINH1 gene in an autosomal recessive. The SERPINH1 gene (located on chromosome 21) is encoding a protein from the serpin superfamily. The serpin, plays a chaperoning role and a key role in the formation of the triple helix of collagen. Mutation in this gene is a determinant of congenital bone fragility found in German Shepherd, English Mastiff and Finnish Lapphund. The aim of the meta-analyses was to determine the interdependence of serpin with other proteins, as well as the co-expression of the SERPINH1 gene with other genes. The following databases and programmes were used in the bioinformatics analyses: NCBI, OMIA, KEGG, STRING v.12.0. The highest combined confidence of the functional interaction of the SERPINH1 protein was estimated with the FKBP10 protein (0.872) and the co-expression score at the mRNA level of the genes encoding these proteins was 0.133. The FKBP10 gene is responsible for encoding a protein that acts as a molecular chaperone. Not much lower predicted functional partners were estimated between the SERPINH1 protein and COL1A1 (0.854) and COL1A2 (0.824). The co-expression score between SERPINH1 and COL1A1 was 0.373, and that of COL1A2 was 0.325. These results indicate a strong correlation between SERPINH1 gene expression and COL1A1 and COL1A2 collagen expression, therefore further studies will focus on the relationship between mutations in these genes and signs of congenital bone fragility in the following breeds: German Shepherd, English Mastiff and Finnish Lapphund. Because of the important role of investigated genes we paid particular attention to the analysis of its mutations. This meta-analysis provided valuable information on molecular mechanisms and provides a basis for developing more effective diagnostic and therapeutic strategies in the domestic dog.

### Search for mutations in selected exons of the SLC3A1 gene sequence associated with cystinuria in Irish Terrier dogs

J. Owczarek<sup>1</sup>, D. Goworek<sup>1</sup>, B. Kominek<sup>1</sup>, B. Grzegorzóka<sup>1,2</sup>, J. Gruszczynska<sup>2</sup>

<sup>1</sup> Warsaw University of Life Sciences – SGGW, Scientific Association of Experimental and Laboratory Animals, Ciszewskiego 8, 02-786 Warsaw, Poland, <sup>2</sup> Warsaw University of Life Sciences – SGGW, Department of Animal Genetic and Conservation, Ciszewskiego 8, 02-786 Warsaw, Poland

Cystinuria is a genetic disease that is characterized by the disrupted transport of cystine and dibasic amino acids (ornithine, arginine and lysine). As a result of defective reabsorption in the proximal tubule of the nephron, cystine cannot pass into the bloodstream. Therefore, its level in urine increases, which may lead to development of urolithiasis. The genetic basis of cystinuria is best understood in humans. In most dog breeds, the mutation that causes the development of cystinuria has not yet been identified. Both in humans and dogs, cystinuria has been associated so far with mutations in the SLC3A1 and SLC7A9 genes. The purpose of this study was to analyze the sequences of exons 2, 4, 5 and 7 of the SLC3A1 gene in search of the mutation responsible for cystinuria in Irish Terrier dogs. Biological material for the study consisted of hair collected from 69 individuals and blood from 8 dogs of Irish Terrier breed (including 4 diseased, 20 probable carriers of cystinuria). DNA was isolated, purified, and then individual exons of the gene under study were amplified and sequenced. Using bioinformatics programs: GeneDoc 2.7, BLASTx, nucleotide sequences were aligned and amino acid sequences were transcribed. The nucleotide sequences of exon 2, 4 and exon 5 showed no variation between individuals and the reference sequence. In exon 7, a synonymous C/G mutation was found at 127 nucleotide positions. Based on the analyses, it was concluded that cystinuria in the Irish Terrier breed is not caused by a mutation within the examined exons of the SLC3A1 gene.

### Session 5

### Theatre 1

#### Livestock grazing : A sustainable landscape designer

T. Glasser<sup>1</sup>, L. Hadar<sup>1</sup>, G. Rosenberg<sup>2</sup>

<sup>1</sup> Ramat-Hanadiv Nature Park, Research, P.O.B 325, 3095202 Zikhron-Ya'akov, Israel, <sup>2</sup> Technion - Israel Institute of Technology, Civil & Environmental Engineering, Kiryat Hatechnion, 3200003 Haifa, Israel

Livestock grazing is part of Mediterranean agriculture for about 10,000 years. In recent decades, livestock numbers have decreased due to social and economic changes, particularly in mountainous areas. This reduction has caused vegetation changes, more shrub encroachment, and an increased risk of wildfires. Grazing influences vegetation structure, microclimates, soil nutrients, and helps limit fire spread by consuming dry herbaceous biomass. This study investigates the effects of different grazing regimes (goats, cattle, and their combination) on the structural dynamics of Mediterranean vegetation in Ramat-Hanadiv Nature Park, located in Israel's Carmel Mountain range, from 2004 to 2022. The park includes areas managed under different grazing practices: cattle grazing, goat grazing, combined grazing, and a control area without grazing. Aerial photographs taken every 2–3 years were used to create classified orthophoto maps (GISPRO software), distinguishing between green patches (shrubs and trees) and brown patches (soil or dry herbaceous vegetation). Goat movement and grazing intensity were monitored via a GPS collar, as they were herded and grazed as one group. This allowed a spatial correlation between grazing activity and vegetation change, while cattle grazed freely in many small groups and were not specifically monitored. Vegetation structure was analyzed using the 'landscapemetrics' R package, based on FRAGSTATS, focusing on cover percentage (CP), patch density (PD), patch size (PS), and edge density (ED). Vegetation cover and patch size were higher in the control area compared to grazed areas. Patch density was highest under combined goat and cattle grazing. Edge density decreased over time in the control area, while grazed areas showed stable or increasing trends, particularly under combined grazing. Analysis of goat GPS data indicated that 40% of vegetation structural changes from closed to open areas were linked to goat grazing ( $P < 0.001$ ). The findings highlight the role of livestock grazing in enhancing habitat diversity, reducing shrub encroachment, and minimizing wildfire risks, demonstrating its value in managing Mediterranean mountain ecosystems.

### How cultivation intensity shapes the net carbon footprint of Mediterranean cow–calf systems

M. F. Lunesu<sup>1</sup>, S. Sechi<sup>1</sup>, G. Pulina<sup>1</sup>, G. Battacone<sup>1</sup>, A. Nudda<sup>1</sup>

<sup>1</sup> University of Sassari, Agraria, Viale Italia 39, 07100 Sassari, Italy

The present study evaluated the net carbon footprint (Net-CFP) of Mediterranean cow–calf production systems, focusing on the influence of cultivation intensity on environmental performance. Eighteen beef cattle farms operating under a grazing-based cow–calf system with an open production cycle were analyzed. Farms were grouped according to cultivation rate (CR) into high-CR systems (>15% cultivated land) and low-CR systems (<15%). High-CR farms had a higher number of cows (48 vs. 33), and a higher stocking rate (0.91 vs. 0.58 Livestock Unit, LU/ha) compared with low-CR farms. Data were collected through on-farm surveys and analyzed using a cradle-to-farm-gate approach, in accordance with ISO 14040 and ISO 14044 standards. The assessment was based on the average of five consecutive production years. Greenhouse gas emissions were expressed as kg CO<sub>2</sub> equivalent (CO<sub>2</sub>e) per kg of live weight (LW) sold from yearling beef, per kg of total live weight (TLW) sold (including cull cows, bulls, and yearlings), and per hectare of utilized agricultural area. Soil carbon sequestration was quantified by accounting for carbon inputs from above- and below-ground biomass residues, manure deposition, and permanent land uses, including agroforestry systems, forests, and Mediterranean scrublands. Gross carbon footprint was lower in low-CR farms compared with high-CR farms (19.80 vs. 26.75 kg CO<sub>2</sub>e/kg LW sold). Enteric methane represented the dominant emission source, contributing 74.2% and 68.3% of total emissions in low- and high-CR systems, respectively. When carbon sequestration was incorporated, Net-CFP was significantly reduced in low-CR farms compared with high-CR farms (-48.02 vs. -7.3 kg CO<sub>2</sub>e/kg LW sold; P<0.01). These results indicate that reduced cultivation intensity enhances carbon sequestration potential and can substantially mitigate greenhouse gas emissions, potentially offsetting the emissions associated with the fattening phase of cow–calf systems. However, this potential is based on farm level estimates and does not automatically translate into certified carbon credits. Therefore, further work is needed to assess eligibility and practical implementation within formal carbon crediting frameworks.

### Session 5

### Theatre 3

### Tree carbon sequestration offsets emissions from hamburger production in agrosilvopastoral farming systems

M. Finocchi<sup>1,2</sup>, F. G. Cella<sup>1,2</sup>, A. Cappucci<sup>3</sup>, R. Villani<sup>3</sup>, A. Mantino<sup>1,2</sup>, M. Mele<sup>1,2</sup>

<sup>1</sup> University of Pisa, DAFE, Via del Borghetto, 80, 56124 Pisa, Italy; <sup>2</sup> Center of Agro-environmental Research “Enrico Anzani”, Via Vecchia di Marina, 56122 Pisa, Italy; <sup>3</sup> Tellus s.r.l., Piazza Sant’Antonio, 56122 Pisa, Italy

Agrosilvopastoral systems provide a range of ecosystem services including carbon sequestration. This study evaluates the environmental impact and carbon balance of hamburger production from two agrosilvopastoral beef cattle farms using a Life Cycle Assessment approach. The analysis covers the entire production chain, from cattle rearing to meat processing into hamburgers. In both farms, the complete beef production cycle was present: from the cow-calf phase to the fattening phase. The functional unit adopted for the downstream phase is a 180 g hamburger, including packaging. Results indicate that global warming potential is the dominant environmental impact category. The carbon footprint of the hamburger ranges from 5.6 to 8.2 kg CO<sub>2</sub> eq, with the farming phase representing the main source of emissions (73–84% of total emissions) mainly due to methane emission from rumen fermentation processes, followed by hamburger production (16–26%) with a marginally contribution of slaughtering and packaging. Carbon sequestration associated with farm forested areas, both grazed and ungrazed, ranges from 0.8 to 1.1 Mg ha<sup>-1</sup> year<sup>-1</sup>. Overall, carbon uptake can substantially reduce the emissions generated along the hamburger production chain, including downstream processes, and in some cases may offset them entirely. These findings indicate that agrosilvopastoral beef farming systems are able to mitigate the climate impact of hamburger production, potentially leading to carbon-neutral products.

### **Innovative Gastronomic Valorization as a Driver of Long-Term Sustainability in Istrian Cattle**

*E. Šuran<sup>1</sup>, A. Ivanković<sup>2</sup>, G. Šubara<sup>1</sup>, M. Pečina<sup>2</sup>*

*<sup>1</sup> Agency for Rural Development of Istria, Ulica prof. Tugomila Ujčića 1, 52000 Pazin, Croatia, <sup>2</sup> University of Zagreb Faculty of Agriculture, Division of Animal Science, Svetošimunska cesta 25, 10000 Zagreb, Croatia*

Experience in the conservation of local breeds in Croatia indicates that long-term sustainability depends on the effectiveness of models aimed at the economic and social repositioning of a breed. This study aimed to evaluate the role of gastronomy as a key driver in optimizing conservation strategies, using Istrian cattle as a representative local breed. The analysis was based on available population indicators, including carcass characteristics and meat quality traits of thirty young Istrian bulls. Carcass conformation and fatness were assessed according to the EUROP classification system, while meat chemical composition was determined using near-infrared transmission spectrophotometry and gas chromatography. Assessment of gastronomic valorisation was further informed by a sociological perspective addressing the historical development of the Breeders' Association. The Istrian cattle conservation program, implemented for more than four decades, comprises three major phases: an initial phase characterized by informal conservation activities undertaken by breeders and the scientific community, supported by international associations, resulting in the establishment of a population inventory and increased public awareness; a second phase focused on breed standardization, the definition of breeding objectives, and the introduction of financial subsidy schemes; and a third, ongoing phase defined by the implementation of in situ and ex situ conservation measures and the economic repositioning of the breed through innovative gastronomic approaches. Although a relatively modest average carcass conformation score (5.34), the meat has high nutritional quality, reflected in protein content (23.73%) and a favourable lipid profile with a high proportion of unsaturated fatty acids (58.03%). Alignment with modern consumer preferences can be effectively achieved through gastronomic innovation, enabling the development of products with higher added value and establishing gastronomic valorisation as a central determinant of the long-term sustainability of Istrian cattle and their breeders.

### Session 5

### Theatre 5

### **Does the extended quality negotiation of Corsican cured pork products help achieve functional integrity?**

*D. Drevon<sup>1,2</sup>, P. Cornu<sup>2</sup>, M. O. Nozières-Petit<sup>1,2</sup>*

*<sup>1</sup> INRAE, UMR Selmet, 2 Place Pierre Viala, 34060 Montpellier, France, <sup>2</sup> INRAE, UMR Territoires, 9 avenue Blaise Pascal, 63178 Aubière, France*

Geographical indications are perceived in Mediterranean countries as privileged tools for reflecting on the sustainability of livestock farming systems. This paper explores this assumption through the concepts of functional integrity, understood as a dynamic vision of sustainability, and extended quality, defined as a form of quality integrating intrinsic and extrinsic dimensions. Using the case of three cured pork products recognised as PDOs since 2014, this study examines the dynamics of free-range pig farming development on the Mediterranean island of Corsica. By negotiating the extended quality of cured pork products, are stakeholders able to build and maintain the functional integrity of farms and their socio-ecosystem over time? This research is based on an interdisciplinary qualitative methodology combining biotechnical analysis of livestock systems (11 farm trajectories) with a socio-historical approach (11 interviews, archives). The results highlight the sequencing of functions at the intersection of the socio-ecosystem, the market and the sociotechnical network. From the 1960s and 1970s onwards, the community-based silvopastoral pig farming system underwent profound transformations. Drawing on increasingly dense local sociotechnical networks, Corsican pig farming systems were analysed and supported, leading to functional integrity in which commercial viability was ensured. Although imperfectly, social and economic activities were also maintained in Corsica's inland areas. However, while efforts devoted to the local pig breed contribute to the environmental function, farmers remain subject to global changes, and current practices are undermining this function through increased dependence on feed inputs. The trajectory of Corsican PDO cured pork products illustrates a dual movement: market integration on the one hand, and specialisation in Mediterranean free-range pig farming on the other. This diachronic analysis suggests that, despite the commercial success of qualifying cured pork products through extended quality, the quality-oriented approach now faces challenges that call into question the functional integrity built since the 1970s.

### Meat quality of Maremmana young bulls reared under grass-fed and intensive systems

S. Tavaniello<sup>1</sup>, M. Peng<sup>1</sup>, D. B. Teshale<sup>1</sup>, M. Palazzo<sup>1</sup>, R. Salvati<sup>2</sup>, G. Senczuk<sup>1</sup>, F. Pilla<sup>1</sup>, G. Maiorano<sup>1</sup>

<sup>1</sup> University of Molise, Agricultural, Environmental and Food Sciences, Via F. De Sanctis, 86100 Campobasso, Italy, <sup>2</sup> Presidential Estate of Castelporziano, Via Pontina 690, 00128 Roma, Italy

After weaning, at approximately 9 months of age, twelve young Maremmana bulls (an autochthonous breed from Tuscany, Italy) were randomly allotted to two rearing systems: grass-fed (GF, on natural pasture) and intensive (IS, mixture of dry corn, bran, straw, soy). Bulls were slaughtered at 16 months of age. Group slaughter weight and individual cold carcass weight were recorded. The Longissimus dorsi samples were collected from the carcass (24h, 2–4°C) for physico-chemical analyses. Data were analyzed by one-way ANOVA. The IS group had a numerically higher slaughter weight (608.2 and 441.7 Kg) and cold carcass weight (331.2±42.56 vs 234.7±13.75; P<0.01). The rearing system did not affect meat pH, color and protein content; meat from the IS group had lower (P<0.01) moisture content and higher (P<0.01) total lipid content compared with the GF group. The fatty acid (FA) profile showed no differences in saturated fatty acids (SFA), the predominant FA class, between groups. Monounsaturated fatty acids (MUFA) were higher in IS meat (P<0.01). This significant increase involved all major MUFAs such as myristoleic (C14:1), palmitoleic (C16:1), and oleic (C18:1) acids, except the trans-vaccenic acid (C18:1 t-11), which was more abundant in meat from the GF group (P<0.01). Total polyunsaturated fatty acids (PUFA), as well as n-6 and n-3 PUFA classes, were higher (P<0.01) in GF beef than in IS beef. Conjugated linoleic acid (CLA, C18:2 c-9, t-11), known for its beneficial effects on human health, was present at a higher level (P<0.01) in GF beef. Likewise, long-chain n-3 PUFAs, such as C20:5, C22:5, and C22:6, were also higher (P<0.01) in GF beef. Furthermore, GF beef showed a nutritionally favorable n6/n3 ratio and a higher PUFA/SFA ratio (P<0.01). No differences were observed in the atherogenic index, whereas the thrombogenic index was lower (P<0.05) in GF beef. In conclusion, beef from grass-fed Maremmana young bulls exhibits a better nutritional profile, characterized by lower lipid content and higher levels of beneficial n-3 PUFA, compared with beef from intensively reared animals.

## Session 5

## Theatre 8

### Natural salt land pasture represents an interesting agroecological feeding alternative for sheep in saline regions

L. Majdoub-Mathlouthi<sup>1</sup>, M. Friha<sup>1</sup>, R. Aloulou<sup>1</sup>, S. Massaoudi<sup>1</sup>, M. Rouatbi<sup>1</sup>

<sup>1</sup> Higher Agronomic Institute of Chott-Mariem, Animal Production, Chott-Mariem, 4042 Sousse, Tunisia

Due to climate change, south coastal mediterranean areas are threaten by the increase of drought, soil salinity and the rise of sea level. An expansion of salt lands characterised by an extension of a large range of wild halophytes is observed. In the archipelago of Kerkennah, situated in Tunisia, these marginal areas are traditionally valorised by sheep as pastoral resources. We aim in this study to synthesize data obtained from three trials realised on saline pastures of the archipelago during three different years (2018, 2029 and 2023). These data concerned intake, growth and carcass performances of lambs. 75 female lambs (Queue Fine de l'Ouest or Queue fine de l'Ouest x Barbarine) of an average weight of 23 kg were reared during spring on salt land, characterised by the presence of shrubby and herbaceous halophyte species and supplemented with moderate concentrate level (300-400 g). Intake and digestibility were measured using total fecal collection and acid Insoluble Ash marker method on 42 randomly selected lambs. They were than slaughtered at an average weight of 31.6 kg. carcass yield and carcass fatness (kidney fat and shoulder composition) were assessed. The metabolizable energy intake (MEI) and crude protein intake (CPI) averaged 12.3± 4.8 MJ/d and 168.2± 63.8 g/d. ADG averaged 150±46 g/d and was correlated to MEI and CPI (P < 0.05). kidney fat (187 g) and the percentage of fat in the shoulder (13.4%) was negatively correlated (P < 0.05) with CPI/MEI ratio. Lamb's performances are acceptable, considering the breed and the arid conditions. The variability observed was associated to pasture quality and nutritive supply, mainly affected by rainfall. Marginal salt land pasture represents an interesting agroecological livestock feeding alternative, that reduce competition with human food and require much less water, in a context of aridity. This study was carried out as part of the HaloSheep project, with partially the support of the PRIMA programme of the European Commission and the financial support of MESRS

### **Grape stem inclusion in the diet of fattening young bulls increased $\alpha$ -tocopherol deposition in meat**

M. Blanco<sup>1</sup>, A. Civera<sup>1</sup>, C. Conesa<sup>1</sup>, J. Pérez-Jiménez<sup>2</sup>, R. Mateos<sup>2</sup>, S. Lobón<sup>1</sup>, I. Casasús<sup>1</sup>, M. Joy<sup>1</sup>

<sup>1</sup> CITA-IA2, Avda. Montañana 930, 50059 Zaragoza, Spain, <sup>2</sup> ICTAN-CSIC & CIBERDEM-ISCHII, C/ José Antonio Novais, 6, 28003 Madrid, Spain

Grape (*Vitis vinifera*) stems, an agro-industrial by-product, can be included in ruminant diets. They have high fibre content and low protein content, similar to straw, but are rich in secondary compounds that may be deposited in beef. Young bulls (162 days old; 173 kg BW, n=24) were fed iso-energetic and iso-proteic concentrates ad libitum for 17-20 weeks. Concentrates contained different proportions of grape stem and barley straw in inverse ratio: 0% stem (6% straw), 3% stem (3% straw) and 6% stem (0% straw). Carotenoids and tocopherols were determined by UPLC, polyphenols and proanthocyanidins by spectrophotometry, and phenolic compounds by HPLC-MS. Grape stem had high concentrations of  $\alpha$ -tocopherol, extractable polyphenols and proanthocyanidins, as well as small amounts of carotenoids, whereas these compounds were almost negligible in straw. In concentrates, carotenoids were detected at low levels, with no differences among treatments. Tocopherol concentrations were similar across concentrates, being  $\gamma$ -tocopherol the most abundant, followed by  $\alpha$ - and  $\delta$ -tocopherol. The inclusion of grape stem resulted in only minor changes in total extractable polyphenols and low levels of non-extractable proanthocyanidins. Gallic acid and kaempferol glucoside were present due to grape stem inclusion, whereas ferulic acid and 3,4-dihydroxybenzoic acid were already present in the 0% stem concentrate. In beef, no carotenoids were detected; only retinol was present, with no differences attributable to grape stem inclusion. Regarding tocopherols,  $\alpha$ -tocopherol was the most abundant, followed by  $\gamma$ - and  $\delta$ -tocopherol, the latter deposited in small amounts. The concentrations of  $\alpha$ -tocopherol increased with the rate of grape stem inclusion,  $\gamma$ -tocopherol tended to increase, and  $\delta$ -tocopherol was unaffected. Total polyphenol concentrations tended to increase with grape stem inclusion, although this was not accompanied by an increase in the detected individual phenolic compounds. In conclusion, dietary inclusion of grape stem in the diet of young bulls enhanced the deposition of  $\alpha$ -tocopherol in beef.

### **From Indigenous Microbiota to Safe Starter Cultures: Safety Assessment of *Lactiplantibacillus plantarum* Isolated from Traditional Fermented Meat Products**

G. Carboni<sup>1</sup>, M. Chessa<sup>1</sup>, N. P. Mangia<sup>1</sup>

<sup>1</sup> Università degli Studi di Sassari, Agraria, Viale Italia 39A, 07100 Sassari (SS), 07100 Sassari, Italy

The quality and safety of traditional Mediterranean products are linked to artisanal processing practices and to the presence of autochthonous microbial communities. These indigenous microorganisms represent a key factor in defining product identity and technological performance. Lactic Acid Bacteria (LAB), and particularly *Lactiplantibacillus plantarum*, are frequently isolated from fermented meat products and play a central role in fermentation dynamics and product characterisation. *L. plantarum* strains isolated from fermented sausages were characterised for key technological traits, with several strains showing favourable responses. For that, these strains were further subjected to safety assessment and evaluation of antimicrobial activity against spoilage and/or pathogenic microorganisms, with the aim of selecting starter candidates combining favourable technological traits with adequate safety guarantees. *L. plantarum* strains were characterised through phenotypic and genotypic approach to assess their antibiotic resistance profiles, evaluating susceptibility against different antibiotic classes and the presence of selected antibiotic resistance determinants using PCR-based methods performed on both genomic and plasmid DNA. In addition, antimicrobial activity was preliminarily screened on selected *L. plantarum* strains showing lower levels of antibiotic resistance. The analysis of *L. plantarum* isolates revealed heterogeneous antibiotic susceptibility profiles. However, the combined methods indicated a low risk of transferable antimicrobial resistance, as resistance determinants were mainly intrinsic or chromosomally encoded and not associated with mobile genetic elements. In addition, the preliminary antimicrobial screening revealed inhibitory activity by some strains against specific pathogenic and spoilage bacteria. Further studies will focus on elucidating these mechanisms. Overall, these results support the selection of autochthonous *L. plantarum* strains as safe starter candidates, highlighting the importance of preserving microbial biodiversity and maintain the typicity and quality of traditional Mediterranean products.

**Extruded linseed for improving milk quality and environmental sustainability in local Sar-do-Bruna cows***F. Correddu<sup>1</sup>, S. Carta<sup>1</sup>, A. Nudda<sup>1</sup>**<sup>1</sup> University of Sassari, Dipartimento di Agraria, viale Italia 39/a, 07100 Sassari, Italy*

The objective of this work was to assess the effect of dietary linseed on the milk quality and production sustainability of Sar-do-Bruna cattle. To this aim, eighteen lactating cows, reared in a private farm, were selected and assigned to two experimental groups: control (CON) and extruded linseed (ELS). The CON was fed a basal diet (8 kg/d of commercial concentrate and mixed hay ad libitum), whereas, the ELS group was fed the same diet where 1 kg of concentrate was replaced by 1.2 kg/head of extruded linseed. During the trial (lasting 4 weeks) individual milk yield was recorded and samples were collected weekly. Milk samples were divided into 3 aliquots and analyzed for milk composition (mid infrared spectroscopy), milk coagulation ability (formagraph), and milk fatty acid profile (gas-chromatography). The environmental impact of production was assessed, directly, by measuring the enteric methane emitted by cows using a laser methane detector and, indirectly, by prediction equations based on milk FA profile. Data were analyzed by a mixed model where diet, time and their interaction were considered as fixed effects, and animal as random effect. Milk yield and composition were not affected by the dietary treatment, except for the FA composition. As expected, the milk of ELS group showed larger concentration in polyunsaturated FA and lower in saturated FA, highlighting an improved quality from nutritional and nutraceutical aspects. No negative effect of the treatment was detected from the cheesemaking perspective. Methane emissions were significantly reduced by the treatment; in particular, the two methods agreed for a reduction of about 15%. In conclusion, the use of extruded linseed in the diet of Sar-do-Bruna cattle could be useful to improve the quality of dairy products and to reduce the environmental impact of the farm.

**Effects of bulk milk quality on Pecorino Romano cheese yield***M. Farina<sup>1</sup>, F. Correddu<sup>1</sup>, G. Pulina<sup>1</sup>, A. Nudda<sup>1</sup>**<sup>1</sup> University of Sassari, Dipartimento di Agraria, Sezione Scienze Zootecniche, Viale Italia, 39, 07100 Sassari, Italy*

Sardinia, with a sheep population of 2,657,409, accounts for approximately 75% of Italy's total sheep milk production. Milk produced by Sarda dairy sheep is entirely destined for cheesemaking, including three Protected Designation of Origin (PDO) cheeses (Pecorino Sardo, Pecorino Romano, and Fiore Sardo) primarily destined for international markets. In recent years, the dairy industry has reported a decline in cheese yield, which has been associated with reductions in milk fat and protein contents. To investigate this phenomenon, monthly (December-July) milk samples collected over a 10-year period from one of the largest sheep cheese factories in Sardinia were analysed to evaluate the effect of total milk fat and protein (TFP) on Pecorino Romano cheese yield. The results showed that although TFP content increased by 12.48% ( $p < 0.0001$ ) between December and July, cheese yield did not increase proportionally. Specifically, a 1% increase in TFP in December resulted in a 1.46% increase in cheese yield, whereas in July, the cheesemaking efficiency of TFP significantly declined by 3.66% ( $p < 0.0001$ ). This evidenced a seasonal reduction in the technological properties of milk components directly related to the evolution of lactation stage and the quantity and quality of available pasture. An equation was also developed to estimate 24-hour cheese yield based on milk fat and protein percentages. The results showed that the cheese yield for 69% was related to milk quality, with milk fat contributing approximately 26% more than protein, and approximately the 31% of the variability was related to the technologies adopted by the dairy. In conclusion, the cheese yield requires not only an increase in milk TFP content but also an improvement in the qualitative profile of them. Effective strategies that should be encouraged include incorporating fat and protein content as selection criteria into genetic selection programs for Sarda sheep, improve animal health monitoring, and implement milk payment systems that effectively recompense high milk quality. Acknowledgements: This work was funded by the Italian National Recovery and Resilience Plan (PNRR), Mission 4 "Education and Research", Component 2 "From Research to Enterprise", Investment 3.3 "Innovative PhD programmes addressing the innovation needs of enterprises and promoting the employment of researchers by companies" (Ministerial Decree No. 630/2024), and co-funded by CAO Formaggi, Sardinia.

**Milk Fatty acid components in Baladi goats reared under various conditions\***

M. Saad<sup>1</sup>, L. Diab<sup>1</sup>, P. Zgheib<sup>1</sup>, R. El Hindi<sup>2</sup>, S. El Murr<sup>3</sup>, K. Houchaymi<sup>3</sup>, K. Jawasreh<sup>4</sup>, S. Sawan<sup>1</sup>, P. Aad<sup>1</sup>

<sup>1</sup> Notre Dame University, Sciences, -, - Zouk Mosbeh, Lebanon, <sup>2</sup> Universite Saint Joseph, ESJAM, -, - Taanayel, Lebanon, <sup>3</sup> Lebanese Agricultural Research Institute, Animal Research Station, -, - Terbol, Lebanon, <sup>4</sup> Jordan University of Science and Technology, of Animal Production, -, - Irbid, Jordan

Driven by the increasing demand for dairy goats' milk for their unique fatty acid composition, this study investigates milk fat composition in Lebanese Baladi goats raised under different management systems for the first time in Lebanon. Milk samples from 184 does raised under intensive, pastoral or agropastoral management were collected twice for each animal (early and late lactation) and analyzed using the Milkoscan to determine the proximate composition (fat, protein, lactose and solids-non-fat). Fatty acid profiles were characterized using Gas Chromatography-Mass Spectrometry (GC-MS) following freeze-drying and methanolysis. Results indicated that while the management system and lactation stage significantly influenced protein, lactose, and SNF levels ( $p < 0.05$ ), total fat content remained unaffected ( $p > 0.05$ ) by either. However, GC-MS revealed a significant interaction between management systems and lactation stage regarding unsaturated fatty acids (UFA) and long-chain saturated fatty acids (LC-SFA) ( $p < 0.05$ ). Specifically, the agropastoral system during late lactation produced the highest quality milk, characterized by elevated concentrations of medium-chain fatty acids and UFAs. Further work will involve conducting a Genome-Wide Association Study (GWAS) to identify genetic markers correlated to the various rearing systems. These findings can be instrumental in developing future selection programs to improve milk quality for premium cheese production. \*Supported in full by Partnerships for Enhanced Engagement in Research (PEER) Program, under USAID, Award Number AID-OAA-A-11-00012. Keywords: Milk fatty acid; Baladi goats; Management systems

**Artisanal fermented Tunisian goat's milk : physico-chemical, microbiological, organic acid composition and volatile compounds**

Z. Jrad<sup>1,2</sup>, A. Chahbani<sup>1</sup>, M. Hammadi<sup>1</sup>, H. El Hatmi<sup>1,2</sup>

<sup>1</sup> Arid Land Institute, Wildlife and livestock laboratory, Road Djorf km 22.5 El Fjè, 4119 Medenine, Tunisia, <sup>2</sup> Higher Institute of Applied Biology of Médenine, Agri-food deptment, Road Djorf km 22.5, 4119 Medenine, Tunisia

Traditional fermented goat's milk "L'ben" from south of Tunisia, is produced through ancestral artisanal practices and represents a particular element of the local dairy heritage. This study provides a comprehensive compositional characterization of "L'ben". To assess the quality of goat dairy products and determine their typical characteristics, samples of raw goat milk and its derivatives (L'ben) were collected, transported under appropriate conditions at 4°C, and then sent to the laboratory. Given the artisanal nature of the production and the sensitivity to changes in milk composition, the quality of the collected goat dairy products is closely linked to the animals' diet, which directly affects milk characteristics such as fat content, protein profile, and volatile compounds. The results demonstrate certain specific characteristics, with a high fat content ( $55.33 \pm 1.52$  g/l) and protein content ( $39.09 \pm 0.63$  g/l) for raw goat's milk, increased acidity ( $75.9 \pm 3.83$  °D), a dominant lactate level ( $5283.351$  mg/l) – indicating significant spontaneous lactic fermentation – and a particular creaminess for L'ben ( $210 \pm 17.32$  cP). Microbiological characterization of the different samples shows that the total bacterial load appears higher in L'ben than in raw milk. This is attributable to the traditional preparation method of L'ben, which uses a Chakoua churning tool made from goatskin. The significant increase in this fungal flora in our L'ben samples, at  $11.51 \times 10^5$  CFU/ml, was observed. Unaffected by acidity and with the residual lactose ( $39.79$  g/l) providing an abundant energy source, they can thrive in the L'ben. Paradoxically, the traditional method can also introduce a diversity of beneficial lactic acid bacteria. In our study, lactic acid bacteria formed the largest group in the L'ben, with a value of  $7.39 \times 10^5$  CFU/ml. Moreover, the key volatile flavor compounds in L'ben were ethyl acetate, acetic acid, hexanoic acid and 2,3-butanedione. Similarly, the hedonic test demonstrated consumer preference for artisanal products.

**Camel milk yogurt fortified with Tunisian Pomegranate (*Punica granatum* L.) syrup and powder**Z. Jrad<sup>1,2</sup>, O. Oussaeif<sup>1</sup>, M. Hammadi<sup>1</sup>, T. Khorchani<sup>1</sup>, H. El Hatmi<sup>1,2</sup><sup>1</sup> Arid land institute/Higher Institute of Applied biology, wildlife and livestock laboratory, Road Djorf km 22.5 El Fjè, 4119 Medenine, Tunisia, <sup>2</sup> Higher Institut of Applied Biology, Agri-food department, Road Djorf km 22.5 El Fjè, 4119 Medenine, Tunisia

Currently, the food industry is compelled to develop new goods that suit the needs of consumers in terms of functional qualities, nutritional value, and therapeutic value. Recent research has looked at the effects of sucrose substitutes on rheological behavior, sensory qualities, and physicochemical features, as well as identifying many dairy product benefits. In this framework, incorporating fruits in the formulation of camel yogurt can contribute to health-promoting camel milk products with high nutritive value. This study was conducted to evaluate the physicochemical and organoleptic qualities of camel yogurt fortified with pomegranates seeds and syrup. In order to reduce added sugar and any other chemical flavoring and coloring agents, three formulations of camel dairy product were prepared with different proportion of pomegranate seeds powder (P) and syrup (S). The first one (F1) contained an equal weight mixture of P and S (P/S=1), F2 was more enriched in S than P (S/P=2); while the last one (F3) was enriched in P (P/S=2). The fraditional ormlulation (T) yogurt without any addition was used as control. Physicochemical (titrable acidity, dry matter, colour, syneresis and viscosity) and sensory properties of different formulations were examined during 21 days at 4°C. The results showed that the formulation of camel yogurt F3 (the richest in powder) had the highest dry matter and mineral content, formulation F2 exuded less water than other formulations, and F1 (P=S) showed the highest viscosity. The incorporation of pomegranate syrup with high proportion offered the most desirable formulation regarding sensory and color evaluation.

**Functioning Principles and Resilience of Agro-pastoral Livestock Systems in Slovenia**D. Bojkovski<sup>1</sup>, T. Flisar<sup>1</sup>, M. Simčič<sup>1</sup><sup>1</sup> Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Agro-pastoral production systems in Slovenia, particularly in Alpine, sub-Alpine, and Karst regions, are mainly based on permanent grasslands used for local breeds adapted to harsh and variable environmental conditions. These systems, traditionally associated with local breeds such as Jezersko-Solčava and Bovec sheep, Cika cattle, and Drežnica goat, combine livestock farming with landscape management, biodiversity conservation, and cultural heritage preservation, making them highly multifunctional. This study analyses how these systems are currently evolving under economic pressures, demographic shifts, and climate change, and identifies their main strengths, weaknesses, and key operating principles. Special attention is given to grazing management, seasonal use of pastures, reliance on local feed resources, and adaptation of breed characteristics to environmental conditions as key factors enhancing system resilience and reducing dependence on external inputs. These features are discussed in the context of agroecological transition, highlighting their potential contribution to climate change adaptation and mitigation through the provision of ecosystem services, carbon storage in permanent grasslands, and reduced reliance on imported feed. The Slovenian case illustrates how traditional agro-pastoral systems could offer transferable experiences for the development of more sustainable and climate-resilient livestock farming models in other regions.

### Rational Grazing Systems in Italy: Toward a Model for Sustainable Development

D. Bochicchio<sup>1</sup>, T. Amoriello<sup>2</sup>, M. Ferrari<sup>2</sup>, S. Santini<sup>1</sup>, M. Scotti<sup>3</sup>, D. Valcavi<sup>1</sup>, E. Zanelli<sup>1</sup>

<sup>1</sup> CREA, ZA, Via Beccastecca 345, 41018 San Cesario sul Panaro (MO), Italy, <sup>2</sup> CREA, AN, Via Ardeatina 546, 00178 Roma, Italy, <sup>3</sup> CREA, PB, Via Barberini 36, 00187 Roma, Italy

Sustainable food systems have increasingly become an EU policy priority. Sustainability is multidimensional encompassing economic, environmental, and social aspects, including animal welfare, food quality, sustainable production chains and human wellbeing. Pasture-based systems can activate a wide range of ecosystem services contributing to food security, sovereignty and human health. Despite livestock farming is often criticized for competing with human food production, ruminants have the unique ability to efficiently convert fibrous resources from grasslands into high-quality food. Dairy grazing pasture increases healthy and sustainable eating models, animal welfare while favoring land stewardship. Field experiments reveal that milk from pasture-fed cows contained 1,53% of  $\alpha$ -linolenic acid, compared with 0,86% in milk produced indoors. Milk color ( $b^*$  index), influenced by carotenoids, riboflavin, and folates, was markedly higher in pasture milk (8.36) than in barn milk (4.88), corresponding to a 71% increase. These results were obtained in the Apennines (1300 m a.s.l.) using a herd of 25 Rossa Reggiana dairy cows. The animals were managed under a rational grazing system of 25 hectares of pasture. Milk with a more favorable nutritional profile may contribute to healthy and sustainable eating patterns, helping to achieve some of the objectives of the National dietary guidelines. Moreover, grazing allows the ruminant to express the species ethogram in accordance with the most recent scientific positions on animal health and welfare. In this framework, permanent grassland and pastures in Italy (about 3.1 million hectares) represent a largely underutilized resource for sustainable livestock production. A wider and more appropriate use of Italian prairies and pastures may enhance local production systems, improve the nutritional quality of foods, increase human intake of essential micronutrients while fostering sustainability. It is evident that a systemic approach is needed to understand the strengths and opportunities of rational pasture-based systems within broader sustainability objectives.

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### Performance of lambs obtained crossbreeding Sardinian ewes with four meat-type breed rams

F. Satta<sup>1</sup>, F. Castangia<sup>1</sup>, A. Spezzigu<sup>2</sup>, A. Cannas<sup>1</sup>

<sup>1</sup> Department of Agricultural Sciences, Viale Italia 39/A, 07100 Sassari, Italy, <sup>2</sup> Embryosardegna, tecnologia, riproduzione e fertilità, Loc. Suiles 1, 07034 Perfugas, Italy

The optimization of terminal crossbreeding strategies is a key tool to improve productivity and sustainability of sheep farming based on local dairy breeds. This study evaluated reproductive performance, growth, carcass traits, and management implications of crosses between Sardinian ewes and four meat-type ram breeds. A trial was conducted on 102 pregnant Sardinian ewes allocated to four groups according to ram breed: Apennines Merinos (S×A; n=31), Lacaune (S×L; n=29), Dorper (S×D; n=27), and Romanov (S×R; n=15). Reproduction was monitored by ultrasonography. Lambs were weighed weekly from birth to slaughter and fed a total mixed ration ad libitum. The S×A ewes showed the highest fertility (91.2%) but also greatest neonatal mortality. The S×L lambs had the highest prolificacy (1.2), while S×D lambs exhibited better neonatal vitality. Singletons were heavier at birth ( $4.44 \pm 0.89$  kg) than twins ( $3.32 \pm 0.80$  kg) and grew faster, regardless of crossbreed, resulting in higher pre-slaughter weights and carcass yield. Males grew faster than females ( $0.23 \pm 0.02$  kg/d vs  $0.19 \pm 0.01$  kg/d). The S×R lambs achieved the highest pre-slaughter weights. The overall values for females were: slaughter weight  $23.62 \pm 0.69$  kg, age  $97.35 \pm 2.16$  d, hot carcass weight  $13.16 \pm 0.32$  kg, yield  $55.64 \pm 0.65\%$ ; those for males were:  $25.13 \pm 1.73$  kg,  $92.44 \pm 4.72$  d,  $13.74 \pm 0.90$  kg,  $54.79 \pm 0.58\%$ . Males particularly excelled, with favorable hot carcass weight and fat cover (3.00). Lumbar measurements and maximum depth at L3–L4 correlated with pre-slaughter weight ( $r=0.42$ ,  $P=0.019$ ;  $r=0.39$ ,  $P=0.030$ ), confirming their usefulness as predictors. Overall, Romanov rams seemed to be well suited for being crossed with Sarda ewes, possibly because the growth of the lambs occurred in a particularly cold winter. Genetic selection should be integrated with proper nutrition, perinatal care, and management practices to maximize productivity while ensuring animal welfare.

### Enhancing meat production and quality of culled Sarda ewes

F. Satta<sup>1</sup>, F. Castangia<sup>1</sup>, A. L. Pinna<sup>1</sup>, C. Caria<sup>1</sup>, A. Ledda<sup>1</sup>, A. Spezzigu<sup>2</sup>, A. Cannas<sup>1</sup>

<sup>1</sup> Department of Agricultural Sciences, Viale Italia 39/A, 07100 Sassari, Italy, <sup>2</sup> Embryosardegna, tecnologia, riproduzione e fertilità, Los. Suiles 1, 07034 Perfugas, Italy

Meat from culled ewes is an underexploited resource in Mediterranean sheep systems due to poor body condition, low carcass conformation, and limited commercial value. Nutritional strategies, including antioxidant supplementation such as vitamin E, may help restore body reserves and improve carcass and meat quality. A feeding trial was conducted on 77 culled Sarda ewes, assigned to three groups: fat ewes fed a maintenance diet (as fed: 400 g/d wheat straw, 400 g/d barley grains, 300 g/d protein and mineral mix), supplemented with 0.6 g/head/day vitamin E (F+E; n=27); lean ewes supplemented with 0.6 g/head/day vitamin E (L+E; n=25), and lean ewes without vitamin E (L; n=25), both receiving the same fattening diet (as fed: 400 g/d grass hay, 900 g/d barley grains, 300 g/d protein and mineral mix). Over 4 weeks, fat ewes slightly lost body weight (-1.91kg), whereas lean ewes maintained or gained weight (+0.16kg L+E; +0.33kg L; P<0.01). The final BCS was higher in F+E (3.58), while among lean ewes L+E had numerically higher BCS than L (3.02 vs. 2.91). BCS variation during the trial was greatest (P<0.01) in L+E (+0.65) compared with F+E (+0.26) and L (+0.34). Ultrasound measurements of subcutaneous fat and Longissimus dorsi depth at thoracic (T12–T13) and lumbar (L3–L4) sites showed greater tissue accretion in L+E, especially for lumbar fat (+1.28mm). At slaughter, carcass yield range was 39.74–41.24% (highest in L+E). Hot carcass weight was higher (P<0.05) in F+E (21.72kg) than lean groups, while L+E (17.33kg) and L (18.31 kg) were similar. Carcass conformation and fat scores were higher in the vit. E supplemented groups: F+E (70%O, 30%P; fat 3.9), L+E (27%O, 73%P; fat 3.2), L (9%O, 91%P; fat 3.0). Short-term fattening improved body reserves and carcass traits of lean ewes. Vitamin E enhanced fat deposition and carcass traits. Ultrasonography effectively monitored *in vivo* tissue development. Average feed cost per day per ewe was 0.40 €/d (F+E), 0.47 €/d (L+E), and 0.37 €/d (L). The higher cost of vitamin E supplemented diets was compensated by better gain and carcass quality.

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### Secondary compounds in beef due to the inclusion of preserved sainfoin during fattening

A. Civera<sup>1</sup>, C. Conesa<sup>1</sup>, J. Pérez-Jiménez<sup>3</sup>, R. Mateos<sup>3</sup>, S. Lobón<sup>1</sup>, M. Joy<sup>1</sup>, D. Villalba<sup>2</sup>, I. Casasús<sup>1</sup>, M. Blanco<sup>1</sup>

<sup>1</sup> CITA-IA2, Animal Science, Avda. Montañana 930, 50059 Zaragoza, Spain, <sup>2</sup> Universitat de Lleida, Animal Production, Avda. Rovira Roure 191, 25198 Lleida, Spain, <sup>3</sup> ICTAN-CSIC & CIBERDEM-ISCI, C/ José Antonio Novais, 6, 28003 Madrid, Spain

Sainfoin (*Onobrychis viciifolia*; SF) was incorporated into the diets of intensively fed cattle during the fattening period in the form of hay or pellets. Sainfoin is a valuable source of secondary compounds; however, preservation may reduce their concentrations. The deposition of carotenoids, tocopherols, and polyphenols in meat may improve meat quality parameters, depending on their dietary intake. Forty Montbeliard young bulls (age: 117 d, body weight (BW): 188 kg) were fed *ad libitum* for 28 weeks with four diets combining two forages (SF hay and barley straw) and two iso-energetic concentrates (C: commercial concentrate; CSF: concentrate containing 15% SF pellet). The resulting diets were C+straw, CSF+straw, C+SFhay, and CSF+SFhay. In feedstuffs and meat, carotenoids and tocopherols were analysed by UPLC, polyphenols by spectrophotometry, and phenolic compounds by HPLC-QTOF-MS. Sainfoin hay showed the highest contents of xanthophylls, total  $\beta$ -carotene, and  $\alpha$ -tocopherol. It also exhibited the greatest concentrations of extractable polyphenols, as well as extractable and non-extractable proanthocyanidins, followed by straw, SF concentrate, and C concentrate. The main phenolic compounds were kaempferol 3-O-rutinoside in SF hay and SF concentrate, p-coumaric acid in straw, and 3,4-dihydroxybenzoic acid in C concentrate. In the meat, retinol was higher in CSF+SFhay bulls than in CSF+straw bulls. The  $\beta$ -carotene levels were higher in SF hay-fed bulls than in straw-fed bulls, regardless of the concentrate. Lutein was detected only in CSF+SFhay meat. Meat from C+SFhay bulls had higher  $\alpha$ -tocopherol concentrations than meat of both groups of straw-fed bulls (CSF+straw and C+straw). The concentrations of total polyphenol and the detected polyphenol metabolites (gallic acid and 3,4-dihydroxybenzoic acid) did not differ among diets. Dietary inclusion of SF hay enhanced the deposition of key antioxidants, such as carotenoids and  $\alpha$ -tocopherol, in meat, without reflecting differences in polyphenol metabolite concentrations.

### **The quality of traditional sheep, goat and cow cheeses from the mountain regions of the Polish part of the Carpathians**

A. Kawęcka<sup>1</sup>, M. Pasternak-Choroszy<sup>1</sup>, I. Peist<sup>1</sup>, R. Garstka<sup>1</sup>, M. Puchala<sup>1</sup>

<sup>1</sup> National Research Institute of Animal Production, Department of Sheep and Goat Breeding, 1 Krakowska St., 32-083 Balice, Poland

Traditional pastoral farming in mountainous areas is still an important element of agriculture in many countries. Extensive ruminant grazing produces healthy, high-quality food and brings ecological benefits. In the Polish Highland region of the Carpathians, Polish mountain sheep, Carpathian goat and the Red-and-White cattle occur. The aim of this study was to assess the nutritional and health-promoting value of cheese made from the milk of local breeds of cows, goats, and sheep. The research material consisted of traditional rennet cheese produced in a farmyard according to a traditional recipe, separately for each species. The animals were kept in Polish Highlands, under a traditional system. For the purpose of this study, milk was obtained during the grazing season in June. Animals were milked twice a day. Milk obtained from two milkings was used to produce cheese, which was vacuum-packed and processed. Differences were found between the varieties in all analyzed characteristics. Goat and sheep cheeses were characterized by higher dry matter, protein, and fat contents than cow cheeses. Sheep cheese contained the most minerals. The caloric and energy value of goat and sheep cheeses was similar and significantly higher than cow cheese. The highest content of caprylic and capric acids was found in goat cheeses. Sheep cheese contained the most unsaturated fatty acids: oleic acid, linoleic acid, alpha-linolenic acid, and CLA isomers. The fat content of cow milk cheeses was characterized by the highest content of saturated fatty acids (SFA). The UFA-SFA and MUFA-SFA ratio was highest in sheep cheeses, and the PUFA-6/n-3 – in goat cheeses. The content of phenolic components varied depending on the species. The FRAP method revealed the highest antioxidant potential in sheep products. Traditional sheep, goat, and cow's milk cheeses from the Carpathian region are characterized not only by their exceptional flavor but also by their high nutritional and health-promoting value. The distinct properties of different milk varieties translate into the nutritional and organoleptic properties of the products obtained from them.

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### **Metabolic properties and functional diversity of the microbial community in Fruhe/Casu axedu during cold storage**

M. Chessa<sup>1</sup>, G. Carboni<sup>1</sup>, N. P. Mangia<sup>1</sup>

<sup>1</sup> University of Sassari, Dipartimento di Agraria, V.le Italia 39, 07100 Sassari, Italy

Sardinian dairy products from sheep and goat milk such as Fruhe/Casu axedu and Gioddu have long attracted scientific and consumer interest due to their contribution to the health of local populations. Traditionally, Fruhe was produced by shepherds for household consumption: raw sheep's or goat's milk was heated and inoculated with an unknown quantity of whey from cheese production. It was then coagulated using lamb rennet paste. Currently, food safety regulations require that milk used to produce Fruhe be pasteurized. The cold chain must also be maintained during storage. The present study aimed to investigate the structure and metabolic activity of microbial community from Fruhe/Casu axedu produced by three producers (A, B and C). To this end, community-level physiological profiling (CLPP) approach was employed to assess carbon source utilization using Biolog EcoPlates. The microbial catabolic potential was determined through Average Well Color Development (AWCD), Richness (R) and Shannon (H) indices. Preliminary results showed that samples from producer A exhibited a progressive increase in AWCD, R and H indices, indicating high metabolic activity and marked functional diversity. In contrast, samples from producer B displayed limited functional diversity throughout storage. Samples from producer C showed high initial values of AWCD, R and H indices, followed by a decline over time, suggesting an early intense microbial activity and a subsequent reduction in community diversity. Patterns of utilization of different carbon source groups (amino acids, carbohydrates, carboxylic acids, amines, and polymers) further highlighted differences in microbial composition, metabolism, and functional behavior of the communities during storage. Overall, the observed differences among producers reflect not only variations in microbial community composition and functionality, but also differences in processing technologies and hygiene practices. These findings indicate that Casu axedu/Fruhe harbors a characteristic and specific microbial ecosystem, which plays a key role in determining the biochemical traits evolution of the product during storage. Acknowledgements: PASAGROPAS-2023CANNAS Project (CUP J83C22002370002).

**Association between milk quality and Somatic Cell Count with Pecorino cheese yield: a retrospective analysis.**

M. Farina<sup>1</sup>, F. Correddu<sup>1</sup>, G. Pulina<sup>1</sup>, A. Nudda<sup>1</sup>

<sup>1</sup> University of Sassari, Dipartimento di Agraria, Sezione di Scienze Zootecniche, Viale Italia, 39, 07100 Sassari, Italy

The efficiency of cheese-making processes is strictly dependent on milk composition. In dairy sheep industry, understanding factors affecting cheese yield is crucial for economic sustainability. This study aimed to assess the effect of milk chemical composition and cellular content on Pecorino cheese yield (CY). The study population was drawn from a comprehensive dataset of 206 dairy sheep farms supplying milk to one of the largest cheese factories in Sardinia, Italy. From this pool, 30 farms were selected and categorized into three groups based on their historical Pecorino Romano cheese yield: High, Medium, and Low. Bulk milk samples were collected monthly (December to July) between 2015 and 2024 to determine composition and SCC. The CY was estimated through a predictive equation based on milk fat and protein content. Consistent with the equation used, farms in the High CY class were characterized by significantly higher levels of fat and protein compared to the Low CY group ( $P < 0.001$ ). Conversely, an inverse relationship was observed for SCC, the High CY class had a significantly lower values than the Low CY class ( $P = 0.01$ ). The High CY group showed lower pH (6.73) and NaCl content (128 mg/100g) than the Low CY group (6.76 and 134 mg/100g, respectively). Data were analyzed using a mixed-effects model. Several parameters showed a significant interaction between CY class and month ( $P < 0.001$ ) except SCC. The differences in milk SCC content remains relatively constant across the months suggesting the presence of persistent mastitis or inflammatory states, as supported by the higher pH and NaCl in the Low CY class. Such physiological alterations negatively impact coagulation properties during the cheese-making process. Namely, while the High Yield group remained distinct, the gap between Medium and Low Yield classes is very close, as evidenced by the lack of significant differences for several parameters. In conclusion, monitoring mammary health, from the start of lactation, are one of the primary strategies for optimizing the cheese-making efficiency of sheep milk into Pecorino cheese. This work was funded by the PNRR (M4C2, Investment 3.3; MD 630/2024) and co-funded by CAO Formaggi.

## Session 5

## Poster 24

**Characterization of milk from Pura Raza Española mare's breed, first trial to valorise the Spanish equine sector**

M. S. Lara<sup>1</sup>, A. Ponzio<sup>2</sup>, M. I. Berruga<sup>1</sup>, A. M. Molina<sup>1</sup>

<sup>1</sup> Universidad de Castilla-La Mancha, IAgri-Food and Industrial Biotechnology Group, Campus Universitario, s/n, 02701 Albacete, Spain, <sup>2</sup> Università Cattolica del Sacro Cuore, Department for Sustainable Food Process, Via Leonida Bissolati 74, 26100 Cremona, Italy

The Pura Raza Española (PRE) horse is a breed of high cultural and economic value in Spain; however, the potential of its milk remains unknown. The aim of this work was the characterization of milk obtained during a lactation from adult PRE mares. Specifically, a comprehensive analysis of the physicochemical and hygienic-sanitary parameters and their evolution throughout the lactation was conducted, with the foundational aim to upgrade the value of the equine sector by exploring the possibilities of incorporating mare's milk into the dairy industry. The study covered the milking period starting from 45 days post-foaling until weaning (6 month). A mechanical milking system was employed, adapting the animals to the routine to minimize stress. Milk yield was recorded every 15 days, and individual samples were collected for the analysis of lactose, protein, fat, solids-non-fat, lactose, acidity, pH, viscosity, colorimetry, somatic cells counts and microbial enumeration using standard dairy analyzers. The average daily yield was 0.64 L/milking. Physicochemical analysis revealed the specific profile of the PRE breed, showing mean values of 7.13 for pH and 5.07 °D for acidity, confirming a composition low in fat and rich in lactose and whey proteins. Bacterial counts indicated a high percentage of lactic acid bacteria relative to the total population, highlighting the potential of this milk for the development of fermented dairy products, the study of which is being carried out. The results will provide a deeper understanding of the PRE breed's characteristics, thus establishing a foundation for its productive potential. These findings suggest the possibility of adding value to this milk in the national dairy market, taking advantage of the nutritional properties described for fermented mare's milk, traditionally consumed in Central Asia.

### Unveiling the potential of enzymatically pre-treated, vacuum paddle-dehydrated tomato pomace as a sustainable food resource for dogs

M. M. Soares<sup>1</sup>, C. Barroso<sup>1</sup>, T. Aires<sup>2</sup>, A. J. Fonseca<sup>1</sup>, A. R. Cabrita<sup>1</sup>

<sup>1</sup> REQUIMTE, LAQV, ICBAS, School of Medicine and Biomedical Sciences, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal, <sup>2</sup> SORGAL, Sociedade de Óleos e Rações S.A., Estrada 109, 3880-728 S. João Ovar, Portugal

Industrial processing of tomato generates large amounts of tomato pomace (TP), whose disposal and stabilization are challenging due to its high moisture content. This study first evaluated the effects of enzymatic pre-treatment of vacuum paddle-dehydrated TP on chemical composition, *in vitro* digestibility using a two-step multienzyme incubation method adapted for dogs, and antioxidant activity, showing improved digestibility. Secondly, effects of enzymatic pre-treatment, vacuum paddle-dehydrated TP (ETP) inclusion in extruded diets were evaluated. Iso-nitrogenous and iso-caloric diets containing 0%, 2% or 4% ETP were formulated. Palatability was assessed using two-bowl tests, and a feeding trial was performed as four 3 × 3 Latin square design with 12 Beagle dogs, three diets and three 28-day periods. Palatability was assessed using chi-square and Student's t-tests, and feeding trial data were analyzed by a mixed model with square, period, and diet as fixed effects, dog within square as random, with orthogonal contrasts for diet effects. Palatability parameters were not affected, but a tendency for a preference for the 2% diet over the 4% diet was observed. Body weight, coat quality, intake, fecal consistency, output, and IgA concentration were unaffected. Fecal dry matter content and pH were lower, and butyrate and valerate proportions were higher, in dogs fed the 2% ETP diet. A linear effect was observed for starch and ether extract digestibility, but all diets exhibited high digestibility (>90%). Overall, ETP represents a sustainable food resource, but further studies evaluating its effects on fecal microbiota and health-related parameters are needed to elucidate its functional potential and address current challenges in tailored companion animal nutrition. Funding: PT national funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through project UID/50006/2025 DOI 10.54499/UID/50006/2025 -Laboratório Associado para a Química Verde – Tecnologias e Processos Limpos.

### Replacing cereals with former food products shifts nitrogen excretion and lowers apparent total tract digestibility in growing-finishing pigs

G. Bee<sup>1</sup>, R. E. Amarie<sup>2</sup>, M. Čandek-Potokar<sup>3</sup>

<sup>1</sup> Agroscope, la tiolèyre 4, 1725 Posieux, Switzerland, <sup>2</sup> University of Pisa, via del borghetto 80, 56124 Pisa, Italy, <sup>3</sup> Kmetijski inštitut Slovenije, Hacquetova ulica 17, 1000 Ljubljana, Slovenia

A metabolic trial quantified nitrogen (N) balance, apparent total tract digestibility (ATTD) of nutrients, and water consumption in growing-finishing pigs fed graded former food products (FFPs). Twenty-four pigs were housed individually in metabolic crates and offered *ad libitum* isonitrogenous, isoenergetic diets containing 0%, 20% or 40% FFPs as a substitute for cereals. Two 6-day total-collection periods were conducted at the end of the grower (~60 kg BW) and finisher (~100 kg BW) phases. Daily feed and water intake were recorded. Total faeces and urine were quantified daily, pooled within pig and period, and analysed for N. Faecal gross energy and phosphorus were analysed to estimate ATTD of energy and phosphorus alongside N. The N intake, excretion, retention (by difference) and N use efficiency (N retained/N intake) were calculated. In the grower phase, FFPs did not affect feed or water intake, but altered N partitioning. Faecal N excretion increased linearly by 34% and 62% at 20% and 40% FFPs (P<0.01), whereas urinary N excretion decreased by 20% and 30% (P<0.05). The N retention and N use efficiency were similar across diets. The ATTD of N, energy and phosphorus decreased linearly with FFPs (P<0.01), with maximal reductions of 12%, 6% and 12%. In the finisher phase, FFPs had no effect on N intake or urinary N losses, but increased faecal N excretion up to 34% (P<0.02) and water intake up to 60% (P<0.01). The ATTD of N, energy and phosphorus decreased linearly (P<0.001), with maximum reductions of 5%, 5% and 12%. In summary, partial cereal substitution with FFP maintained feed intake but reduced ATTD of N, energy and phosphorus and shifted N from urine to faeces. This is environmentally favourable because urinary urea rapidly hydrolyses to total ammoniacal nitrogen (TAN, NH<sub>4</sub><sup>+</sup>+NH<sub>3</sub>), increasing ammonia volatilisation risk, whereas faecal N contains little urea and requires microbial proteolysis and deamination to generate TAN, slowing formation and reducing the pool of volatilised N during housing and storage. Funding: EU H2020—PIGWEB (Grant Agreement No. 101004770).

**Post-absorptive nitrogen utilisation in pigs drives lower ammonia potential with low-protein diets**A. Antonacci<sup>1</sup>, P. Silacci<sup>2</sup>, G. Bee<sup>1</sup><sup>1</sup> *Agroscope, Pig Research Group, la tiolèyre 4, 1725 Posieux, Switzerland*, <sup>2</sup> *Agroscope, Animal Biology Group, la tiolèyre 4, 1725 Posieux, Switzerland*

Pig production is scrutinised for nitrogen (N) losses and ammonia (NH<sub>3</sub>) emissions. Because excess dietary crude protein (CP) and amino acid supply reduce N utilisation and increase excretion, we tested low-protein (LP) diets in growing-finishing pigs under contrasting hygiene management. Forty-eight Swiss Large White female pigs (20 ± 2.2 kg BW) were housed under good hygiene (control) or poor hygiene (less stringent pen-cleaning protocol) and fed standard (ST) or LP diets. The ST grower/finisher diets were isocaloric with 16.4/12.5% CP. The LP diets had reduced CP, Lys, Met, Thr, Trp and Val by ~20%. Individual intake was recorded via automatic feeders. Spot faeces and urine samples were collected to assess N apparent total tract digestibility (ATTD), faecal and urinary N concentrations, urinary N:creatinine ratio, and in vitro NH<sub>3</sub> production from slurry. The carcass N deposition efficiency was estimated using dual-energy X-ray absorptiometry. Data were analysed using linear mixed models (R v4.4.1) with diet, hygiene and diet×hygiene as fixed effects and litter as a random effect. Hygiene did not affect total N intake, whereas LP pigs consumed 13.4% less N than ST pigs (P < 0.001). Although N ATTD was 3.1% lower in LP pigs, faecal and urinary N concentrations were 16.9% and 25.9% lower, respectively (P < 0.001). Urinary N concentration tended to be higher under good hygiene (P = 0.071), whereas urinary N:creatinine was lower in LP pigs (-37.8%) and under good hygiene (-14.6%) (P ≤ 0.007). In vitro NH<sub>3</sub> production decreased in slurry from LP pigs (-24.9%) but increased under good hygiene (+18.0%) (P ≤ 0.043). Nitrogen deposition efficiency was 10.4% higher in LP pigs and 5.1% higher under good hygiene (P ≤ 0.019). Overall, reduced N losses and NH<sub>3</sub> production potential are driven by improved post-absorptive N utilisation rather than N digestibility per se. LP diets increased N deposition efficiency and reduced urinary N losses and in vitro NH<sub>3</sub> production, while manure NH<sub>3</sub> production potential was influenced independently by hygiene management.

## Session 6

## Theatre 4

**Carcass traits and meat quality of Piedmontese bulls fed with a dietary inclusion of hazelnut skins**E. Diaz Vicuna<sup>1</sup>, A. Brugliapaglia<sup>2</sup>, J. Mota-Gutierrez<sup>1</sup>, K. Srikanthithasan<sup>1</sup>, C. Bianchi<sup>1</sup>, V. Zambotto<sup>1</sup>, E. Fiorilla<sup>1</sup>, B. Quaglino<sup>1</sup>, C. Forte<sup>1</sup><sup>1</sup> *University of Turin, Department of Veterinary Sciences, Largo Paolo Braccini 2, 10095 Grugliasco, Italy*, <sup>2</sup> *University of Turin, Department of Agricultural, Forest and Food Sciences, Largo Paolo Braccini 2, 10095 Grugliasco, Italy*

Future survival of European beef sector lies in the development of innovative strategies capable of increasing its sustainability and quality standards. Among them, the incorporation of agro-industrial by-products such as hazelnut skins (HS) as alternative feed ingredients has recently drawn attention. HS are a polyphenol-rich co-product obtained from one of the most relevant industries of the Mediterranean area. To assess the effects of dietary HS inclusion on beef cattle, the BEEF-HAZE project was developed, performing in vivo and postmortem evaluations of animal health, performance, meat quality and chemical profile. The present study aims to highlight results on carcass traits and quality of beef obtained from cattle fed with HS. An experimental trial involving 80 beef bulls was conducted. Animals were split into a control (C) and a test (T) group and fed iso-energetic and iso-nitrogenous diets, with the T group presenting an 8% HS inclusion. Following a 7-months trial (5-m fattening, 2-m finishing), all animals were slaughtered, and carcass weight (CW) was recorded. Dressing % was calculated in relation to CW and at-slaughter body weight of the live animal; 24h postmortem, individual samples of L. dorsi were collected from 48 carcasses and subjected to weight, pH and color measurement. After 7-days aging, samples were weighed again to determine purge loss, physicochemical characteristics, texture traits, and histochemical evaluations of fibers profile. Data were analyzed through a mixed-model ANOVA, with animal as random effect, and no differences were found among the two groups for any of the parameters in study. Despite a lack of significance from a statistical standpoint, the present results are highly relevant in practical terms. Not only do they confirm findings related to the in vivo parameters, but also further support the hypothesis that including HS in beef cattle diets can represent a sustainable strategy and model of circular economy for Mediterranean livestock systems.

### **Agro-industrial by-products as sustainable feed ingredients: effects of pomegranate and hazelnut supplementation on dairy sheep milk quality**

A. Vastolo<sup>1</sup>, A. Bosco<sup>1</sup>, L. Rinaldi<sup>1</sup>, G. Policastro<sup>2</sup>, M. Fabbri<sup>3</sup>, M. I. Cutrignelli<sup>1</sup>

<sup>1</sup> University of Napoli Federico II, Veterinary Medicine and Animal Production, via Federico Delpino 1, 80137 Napoli, Italy, <sup>2</sup> Telematic University Pegaso, Department of Engineering and Computer Science, Via Santa Chiara 49/c, 80132 Napoli, Italy, <sup>3</sup> University of Napoli Federico II, Department of Civil, Architectural and Environmental Engineering, Via Claudio 21, 80125 Napoli, Italy

The increasing environmental impact and cost of conventional feed ingredients such as maize and soybean meal have stimulated interest in agro-industrial by-products as sustainable alternatives in ruminant nutrition. This study evaluated the effects of pomegranate (PG) and hazelnut (HZ) by-product supplementation on milk yield, composition, and fatty acid profile in dairy sheep. Thirty animals were enrolled and allocated to three groups (10 animals per group): a control group (CTR) fed a conventional concentrate, and two experimental groups receiving a concentrate in which pelleted pomegranate seed and pell and hazelnut skin were included at 40 g/head/day. The forage component of the diet consisted of pasture grazing. Supplementation lasted four weeks and milk composition was analyzed weekly on pooled samples from each group. Data were analyzed using a two-factor ANOVA including diet, time, and their interaction. Milk yield was not significantly affected by dietary treatment (mean values, CTR: 1.59 L/day; PG: 1.74 L/day; HZ: 1.78 L/day). Compared with CTR, milk fat increased from 6.90% to 7.48% and 7.12% in PG and HZ groups, respectively ( $P < 0.001$ ), while protein content increased from 5.99% to 6.71% (PG) and 6.29% (HZ). Milk urea concentration was reduced by PG and HZ supplementation (38.9 and 38.3 mg/dL) compared with CTR (42.7 mg/dL). The HZ group showed a lower proportion of saturated fatty acids (64.7%) compared with CTR (68.5%) and a higher proportion of monounsaturated fatty acids (27.7% vs. 25.0%;  $P < 0.001$ ). Polyunsaturated fatty acids were slightly reduced in both supplemented groups. In conclusion, pomegranate and hazelnut by-products improved milk composition and fatty acid profile without affecting milk yield, supporting their use as sustainable feed ingredients in dairy sheep systems.

### Session 6

### Theatre 6

### **Effect of dietary winemaking by-product on milk fatty acid profile and methane emissions in Sarda dairy sheep**

A. Nudda<sup>1</sup>, S. Carta<sup>1</sup>, R. Steri<sup>2</sup>, D. Meo Zilio<sup>2</sup>, F. Correddu<sup>1</sup>, G. Pulina<sup>1</sup>

<sup>1</sup> University of Sassari, Agricultural, Viale Italia, 39, 07100 Sassari, Italy, <sup>2</sup> Council for Agricultural Research and Economics (CREA), Research Centre for Animal Production and Aquaculture, Via Salaria, 31, 00015 Monterotondo, Italy

This study investigated the potential of grape pomace (GP), a winery byproduct, as a sustainable dietary supplement for lactating ewes, focusing on its impact on production performance, milk fatty acid (FA) composition, and enteric methane (CH<sub>4</sub>) emissions. Twenty-four Sarda ewes were balanced into three groups (n=8): a control group (CON) fed a basal diet, and two experimental groups supplemented with either 100 g/d (GP100) or 150 g/d (GP150) of GP. The trial lasted 6 weeks. Milk yield and quality were monitored weekly. During the final week, CH<sub>4</sub> emissions were quantified using a Laser Methane Detector (LMD) during milking sessions (2 min/ewe) over three consecutive days. GP supplementation did not alter milk yield or gross composition. However, GP inclusion significantly enhanced the nutritional value of the milk lipid fraction. Supplemented groups showed a reduction in saturated FAs and an increase in total unsaturated and monounsaturated FAs compared to CON. Specific beneficial FAs followed a dose-response trend: oleic acid was highest in GP100, while C18:1 trans11 and C18:2 cis9,trans 11 (CLA) reached their peak in GP150 group ( $P < 0.05$ ). Regarding environmental impact, while GP100 showed a numerical decrease in CH<sub>4</sub> concentration during eructation, no statistically significant differences were observed for respiration or eructation peaks across treatments. In conclusion, supplementation of grape pomace into the diet of dairy sheep represents an effective strategy to improve FA profile without affecting production. The lack of a strong anti-methanogenic effect suggests that higher doses or different processing of the pomace might be necessary to significantly mitigate enteric emissions. Acknowledgements: This work was supported by the European Union – Next-GenerationEU (PNRR) through two grants: the Agritech National Research Center (Project CN00000022); and the University of Sassari Progetti di Ricerca Interdisciplinari (DM 737/2021, 2021-2022 resources).

### Effects of feeding time on milk production and composition in Sarda ewes

F. Castangia<sup>1</sup>, M. A. Porcu<sup>1</sup>, A. Ledda<sup>1</sup>, A. S. Atzori<sup>1</sup>, A. Cannas<sup>1</sup>

<sup>1</sup> University of Sassari, Department of Agricultural Sciences, Viale Italia, 39a, 07100 Sassari, Italy

In dairy sheep, milk fat content is markedly lower in morning than evening milking, even with equal 12-hour milking intervals. This may depend on different precursor availability, as feed intake is typically higher during daytime. This study evaluated feeding time effects on milk fat and protein in morning and evening milkings of dairy sheep. The experiment lasted 61 days (April 17–June 16, 2025), including 19-day adaptation to 12-hour intervals (milking: 07:00 and 19:00) and 42-day experimental period. Twenty-one mid-lactation Sarda ewes were divided into three groups (n=7) with ad libitum feeding in different times: DAY (07:00-19:00), NIGHT (19:00-07:00), and CONTROL (24-hour access), balanced for body condition score (BCS), body weight (BW), milk yield (MY), dry total mixed ration intake (TMR), and milk fat. All received identical diets: ad libitum chopped dry TMR whit 88% dry matter (DM) plus 50g beet pulp and soybean meal per milking. Data were analyzed with ANCOVA. Total DMI was significantly lower in NIGHT group compared to DAY and CONTROL (2173, 2398, 2492 g DM/d, respectively; P<0.001). NIGHT ewes consumed larger amounts in shorter periods, particularly between 19:00-21:00. Daily MY was higher in CONTROL than DAY and NIGHT (1942, 1756, 1770 g/d, respectively; P<0.001). Both morning MY (980, 915, 932g, CONTROL, DAY and NIGHT, respectively; P=0.003) and evening MY (962, 840, 837g, CONTROL, DAY and NIGHT respectively; P<0.001) were higher in CONTROL. Morning milk fat concentration was higher in NIGHT than DAY and CONTROL (6.54, 5.89, 5.83%, respectively; P<0.001), with correspondingly higher fat yield (P<0.001). Conversely, evening fat concentration was higher in DAY than NIGHT and CONTROL (6.43, 5.97, 6.25%, respectively; P<0.001). Morning protein was higher in NIGHT than DAY and CONTROL (5.15, 5.01, 4.87%, respectively; P<0.001); evening protein was numerically higher in DAY compared to NIGHT and CONTROL (5.03, 4.99, 4.97%, respectively; P=0.066). Feeding time can significantly impact milk fat and protein content at equal yields per milking, likely due to precursor availability before milking. Strategies to increase morning milk components should stimulate adequate nighttime and early morning feed intake.

## Session 6

## Theatre 8

### Ruminal fermentation of pasture-based diets from agroforestry and conventional systems: a Rusitec study

G. Foggia<sup>1</sup>, T. Malizzi<sup>1</sup>, M. Finocchi<sup>1</sup>, F. Cella<sup>1</sup>, A. Silvi<sup>1</sup>, A. Ripamonti<sup>1</sup>, L. Turini<sup>1,2</sup>, A. Mantino<sup>1,2</sup>, M. Mele<sup>1,2</sup>

<sup>1</sup> University of Pisa, Dipartimento di Scienze Agrarie, Alimentari, Agro-ambientali, via del borghetto 80, 56124 Pisa, Italy; <sup>2</sup> University of Pisa, Centro di Ricerche Agro-ambientali "E. Avanzi", via Vecchia di Marina 2, 56122 Pisa, Italy

Pasture quality is a key determinant of productivity in grassland-based livestock systems and is increasingly influenced by climate changes. Agroforestry systems may mitigate climate-related constraints while enhancing carbon sequestration and other ecosystem services. Although previous studies based mainly on chemical composition suggest improved nutritional traits in forages grown under tree cover, their feeding values remain poorly characterized. This study aimed to compare the ruminal fermentative characteristics and gas profiles of herbage grown in an agroforestry or on an open pasture field. The experiment is based on two Rusitec runs carried out using a rumen simulation technique with six vessels per run and a 10-day fermentation period. Diets consisted exclusively of freeze-dried forage mass harvested from experimental fields in Pisa (Italy) during September 2025. Rumen inoculum was collected at a slaughterhouse from grass-fed beef steers. Daily measurements included pH, redox potential, volatile fatty acids (VFA), total gas production, gas composition, and nutrient degradability. Data from the last five days of incubation, considered the experimental period, were analysed using a linear mixed model. Preliminary results from the first run indicate that the agroforestry pasture exhibited greater overall degradability (+13%) of dry matter compared with the open pasture, as supported by greater total gas production (+18%). This response was mainly associated with increased methane concentration (+34%), notably without a concomitant increase in hydrogen. While pH and redox potential were not affected by treatment, differences in VFA profiles were observed: higher propionate and butyrate molar proportions (+14 and 33%, respectively) and lower valerate production in the agroforestry pasture further suggest qualitative differences in nutritional value compared with the conventional system, probably due to higher fibre degradability.

### Comparison of enzymatic methods for estimating organic matter digestibility in forages

E. Carboni<sup>1</sup>, E. Manzocchi<sup>2</sup>, P. Silacci<sup>3</sup>, V. Novara<sup>1</sup>, V. Z. Grilli<sup>1</sup>, C. Mastroeni<sup>1</sup>, A. Gallo<sup>1</sup>

<sup>1</sup> Università Cattolica del Sacro Cuore, facoltà di Scienze Agrarie, Alimentari e Ambientali, Department of Animal Science, Food and Nutrition (DIANA), Via E. Parmense, 29100 Piacenza, Italy, <sup>2</sup> Agroscope, Ruminant Nutrition and Emissions, Rte de la Tioleyre 4, 1725 Posieux, Switzerland, <sup>3</sup> Agroscope, Animal Biology, Rte de la Tioleyre, 1725 Posieux, Switzerland

The precise and accurate estimation of organic matter digestibility (OMd) is essential for the nutritional evaluation of ruminant feed. Usually, in situ techniques are considered reference methods, but their application is limited by costs, time requirements and ethical concerns, highlighting the need for alternative approaches such as enzymatic methods. This study aimed to evaluate and compare different enzymatic methods for estimating OMd in forages, identifying reliable techniques suitable for routine laboratory analyses. Six forage samples (i.e., hay, grass silage, 3 sorghum silage, and maize silage) were analyzed using two enzymatic methods set for OMd evaluation: i) the French INRA method (Aufrère et al., 2007) and ii) the German Enzymatically Soluble Organic Matter (ELOS) method, derived from De Boever et al. (1986). In parallel, enzymatic methods developed by Gallo et al. (2017, 2018, 2019) were applied to determine the degradability of individual nutrients (NDF, protein and starch). For the latter methods, total tract OMd was estimated from digestible nutrient fractions, applying specific coefficients to predict post-ruminal digestion. A linear relationship was observed between INRA and ELOS estimates ( $R^2 = 0.845$ ; RMSE = 3.65%), with ELOS values slightly overestimated OMd with respect to INRA. The OMd estimated from nutrient-specific enzymatic degradability showed relationships with both INRA ( $R^2 = 0.9914$ ; RMSE = 1.93%) and ELOS ( $R^2 = 0.9956$ ; RMSE = 2.58), confirming the robustness and consistency of here proposed nutrient-based estimation. Overall, the enzymatic estimation of OMd can be considered a valid alternative to in situ methods requiring use of fistulated animals, thus supporting the application of aforementioned methods in reliable and practical tool for routine forage evaluation.

## Session 6

## Theatre 11

### The role of rumen fluid adaptation: an in vitro comparison of diet-adapted and non-diet-adapted rumen fluid for continuous CO<sub>2</sub> and CH<sub>4</sub> monitoring with Gas Endeavour

R. Gannuscio<sup>1</sup>, G. Maniaci<sup>1</sup>, D. Gambino<sup>1</sup>, M. Todaro<sup>1</sup>

<sup>1</sup> University of Palermo, Department of Agricultural, Food and Forestry Science (SAAF), Viale delle Scienze 13, 90128 Palermo, Italy

In vitro gas production techniques, such as the Gas Endeavour system, are widely used to assess rumen fermentation kinetics, providing an effective tool to evaluate rumen function and possible methane mitigation strategies. Donor animal diet and forage characteristics shape rumen microbiota, fermentation, pH, microbial activity, and gas production in vitro. The study aimed to evaluate the effect of ruminal fluid (RF), adapted or non-adapted to the four experimental diets used in the in vivo trial, on in vitro fermentation parameters. All diets, formulated to have the same NDF and net energy, included 2000 g/head/d of hay, while varying concentrate and prickly pear peel silage: the control diet (CTR) contained 450 g/head/d of concentrate; diet A included 300 g/head/d of concentrate + 500 g/head/d of prickly pear peel silage; diet B included 150 g/head/d of concentrate + 1000 g/head/d of silage; diet C included 1500 g/head/d of silage. Diets (3 replicates) were incubated for 24 hours using the Gas Endeavour with two rumen inocula collected via an oro-esophageal probe: adapted rumen fluid (ARF) from 2 sheep fed each experimental diet, and common rumen fluid (CRF) from 2 sheep fed hay and concentrate, managed under identical conditions. A 2-way ANOVA (diet and RF as fixed factors) was used. Methane production in 24 h did not differ among diets, whereas total gas production was affected by both diet and RF: CTR diet produced the highest total gas, while ARF resulted in higher total gas production than CRF (276 vs. 267 mL/3g incubated feed). RF type influenced early gas production kinetics (first 7 h), after which curves converged. During this early phase, total gas and methane production were higher with CRF, whereas ARF reduced both parameters, particularly in diets B and C with highest silage inclusion. These findings show that dietary adaptation of the rumen microbiome significantly affects early fermentation, reducing total gas and methane production in initial stages, and highlight the necessity of using diet-adapted rumen fluid in in vitro studies for accurate and biologically meaningful results.

### Grape pomace as ingredient for extensively reared Churra lambs: effect on animal performance and fatty acid content

S. E. Charef<sup>1</sup>, C. Vieira<sup>1</sup>, B. Martínez<sup>1</sup>, J. J. García-García<sup>1</sup>, A. Benito<sup>1</sup>, S. Olmedo<sup>1</sup>, R. Bodas<sup>1</sup>

<sup>1</sup> Agrarian Technological Institute of Castille and Leon, Livestock Research, Ctra. Burgos, km 119, 47071 Valladolid, Spain

Grape pomace (GP), a wine-industry by-product rich in fibre and antioxidants, can be included in lamb diets, potentially improving meat quality, although it may impair feed digestibility and animal performance. While several trials have been conducted in intensively reared lambs, the effects of GP supplementation in lambs reared outdoors on pasture rich in polyunsaturated fatty acids have not been investigated. An experiment was conducted using 20 Churra lambs. Lambs remained with their dams for 60 days after lambing and were subsequently reared separately until slaughter at 100 days of age. All animals were reared outdoors on natural pastures during the day and housed indoors at night, when they received a mixture of barley, oat and pea grains. Upon arrival at the barn, lambs were divided into two groups: a control group receiving the grain mixture without GP, and a treatment group receiving the same diet supplemented with 20% GP. Once obtained, carcasses were weighed, jointed and evaluated for weight, measurements, conformation and fatness. Meat samples from the Longissimus dorsi muscle were collected for fatty acid analysis. No differences were observed in daily weight gain or in the main carcass characteristics. Only trends towards significance ( $P < 0.10$ ) were detected for leg weight, which was lower in GP-supplemented lambs, and breast weight, which was higher compared to the control group. Regarding fatty acid composition, saturated fatty acids tended ( $P < 0.10$ ) to be higher in GP-supplemented lambs. Overall, GP supplementation had no apparent effects on animal performance or carcass quality. Only subtle changes in fatty acid composition were observed, which will be further explored together with other parameters related to meat quality. Acknowledgements: Project PCI2023-143417 (“The making of fragile agro-ecosystems productive, adaptive and sustainable: multifunctional agro-pastoralism”), funded by MCIN/AEI/10.13039/501100011033 and the European Union (PAS-AGRO-PAS, PRIMA/0014/2022).

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### Effects of partially replacing dehydrated lucerne with almond hulls on lamb growth performance and carcass traits

K. Paulos<sup>1,2</sup>, C. Mestre<sup>1,3</sup>, A. P. V. Portugal<sup>1</sup>, J. Costa<sup>1</sup>, M. T. P. Dentinho<sup>1,4</sup>, O. Gerreiro<sup>5,6</sup>, A. Belo<sup>1</sup>, E. Jerónimo<sup>5,6</sup>

<sup>1</sup> INIAV, Fonte Boa, 2005-424 Santarém, Portugal, <sup>2</sup> Geobiotec, FCT-UNL, 2829-516 Caparica, Portugal, <sup>3</sup> LEAF, ISA, 1349-017 Lisboa, Portugal, <sup>4</sup> CIISA, UNL, 1300-477 Lisboa, Portugal, <sup>5</sup> CEBAL, IPBEJA, 7801-908 Beja, Portugal, <sup>6</sup> MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, & CHANGE – Instituto para as Alterações Globais e Sustentabilidade, 7801-908 Beja, Portugal

Sustainable livestock production increasingly relies on agro-industrial by-products. This study evaluated the impact of partially replacing dehydrated lucerne with almond hulls (AH) on the growth performance and carcass quality of finishing lambs. Thirty-two lambs ( $19.8 \pm 1.73$  kg) were assigned to four diets with 0, 10, 15, and 20% AH, replacing lucerne in a diet composed of 60% of concentrate and 40% of Lucerne. The trial lasted 29 days after 15 days of adaptation. The feed was offered ad libitum. At slaughter, hot (HCW) and cold (CCW) carcass weights were recorded, and carcasses graded using European systems. Data were analyzed using SAS PROC MIXED with orthogonal contrasts. AH inclusion showed a quadratic effect on slaughter liveweight ( $P=0.041$ ), with weights increasing up to the 10% and 15% inclusion levels and decreasing at the 20% level. A similar trend ( $P<0.06$ ) occurred for HCW and CCW, favoring these intermediate AH levels. Average daily gain (306 g/d) and dressing percentage (49.3%) were unaffected ( $P>0.05$ ). Although AH reduced diet costs (€343 to €310/t), the feed cost per kg of gain remained similar. In the 15% AH group, 87.5% of carcasses exceeded 13 kg, with 62.5% graded R (Good) and 50% in classes 3-4 for fat cover. Replacing lucerne with AH up to 15% is a viable strategy, optimizing performance and carcass quality. Acknowledgements: Project “CAPOTACIRCULARFEED – Pilot project on the use of almond hulls in animal feed” (PL24 – 00051), funded by the PROMOVE Program – The Future of the Interior of “la Caixa” Foundation, in collaboration with BPI and FCT; R&D units MED (<https://doi.org/10.54499/UID/05183/2025>) and CIISA (<https://doi.org/10.54499/UID/00276/2025>), and the Associate Laboratory CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>).

### Nutritional composition of almond hulls produced in southern Portugal

E. Jerónimo<sup>1,2</sup>, L. Cachucho<sup>1,2,3,4</sup>, K. Paulos<sup>5,6</sup>, O. Guerreiro<sup>1,2</sup>, M. T. Dentinho<sup>3,4,5</sup>

<sup>1</sup> CEBAL, IPBEJA, 7801-908 Beja, Portugal, <sup>2</sup> MED – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, & CHANGE – Instituto para as Alterações Globais e Sustentabilidade, 7801-908 Beja, Portugal, <sup>3</sup> CIISA, UNL, 1300-477 Lisboa, Portugal, <sup>4</sup> Laboratório Associado para a Ciência Animal e Veterinária, (Al4animals), 1300-477 Lisboa, Portugal, <sup>5</sup> INIAV, Fonte Boa, 2005-424 Santarém, Portugal, <sup>6</sup> Geobiotec, FCT-UNL, 2829-516 Caparica, Portugal

Almond production is increasing worldwide, including in Portugal, leading to greater availability of byproducts such as almond hulls (AH), which represent about 50% of the fresh almond weight. This byproduct has potential for use in animal feed; however, information on the composition and nutritional value of AH produced in Portugal remains limited. To improve knowledge of AH produced in Portugal, 17 samples collected in 2023 from orchards in the Alentejo region were physically and nutritionally characterized. For each sample, the proportion of pure AH and other fractions was assessed, and the total sample was characterized in terms of dry matter (DM), ash, fiber, crude protein (CP), ether extract, sugar, gross energy, and in vitro organic matter digestibility (OMD) determined using the Tilley and Terry method. Pure AH proportion ranged from 80.7 to 99.3%, with over half the samples containing more than 10% impurities. AH showed wide variability in composition, particularly in dry matter (38.7–92.0%), ash (5.83–22.0% DM), sugar (12.2–42.4% DM), fiber (10.1–19.9% DM), CP (2.85–7.48% DM), ether extract (0.45–2.55% DM), and gross energy (3370–4158 kcal/kg DM). OMD ranged from 43.7 to 65.8%. This variability limits the predictability of their nutritional value, highlighting the need for prior characterization before use in animal feeding. Acknowledgements: CAPOTACIRCULARFEED – Pilot project on the use of almond hulls in animal feed (PL24 – 00051), funded by "la Caixa" Foundation, in collaboration with BPI and FCT; R&D units MED (<https://doi.org/10.54499/UID/05183/2025>) and CIISA (<https://doi.org/10.54499/UID/00276/2025>); and Associate Laboratories CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>) and Al4animals (<https://doi.org/10.54499/LA/P/0059/2020>).

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### Replacement of soybean oil with black soldier fly larvae (*Hermetia illucens*) oil in post-weaning piglet diets: effects on performance and blood parameters

M. Parreiras<sup>1,2,3,4</sup>, D. Monteiro<sup>1,2,4</sup>, V. Pinheiro<sup>1,2,4</sup>, O. Moreira<sup>3,4</sup>

<sup>1</sup> UTAD, 72 Department of Animal Science, University of Trás-os-Montes and Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal, <sup>2</sup> UTAD, Center for Animal Science and Veterinary Medicine, Quinta de Prados, 5000-801 Vila Real, Portugal, <sup>3</sup> INIAV, Animal Production, Avenue Professor Vaz Portugal, 2005-424 Santarém, Portugal, <sup>4</sup> Al4Animals, Animal Production, Faculty of Veterinary Medicine, 1300-477 Lisboa, Portugal

Animal production seeks sustainable strategies to improve efficiency, and insects may contribute to this goal. Weaning is a critical period for piglets, frequently associated with post-weaning diarrhea caused by *Escherichia coli*. BSF larvae have been studied as feed ingredients due to their nutritional value and bioactive compounds with antimicrobial potential. The objective of this study was to evaluate the effects of replacing soybean oil (SO) with BSF larvae oil (BSFO) in post-weaning piglet diets. The experiment included two dietary treatments: 1) Control (SO), a diet containing soybean oil, and 2) BSFO, a diet containing BSF larvae oil. Diets were formulated to be isoproteic and isoenergetic across all feeding phases. A total of 48 piglets ((LW×LR)×Pi) were randomly allocated to the two treatments and housed in pairs in 24 rearing pens for seven weeks. Piglets were weighed weekly, and feed intake was recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). No significant differences were observed between treatments for initial body weight (7.146 vs. 6.846 kg; P=0.2382), final body weight (29.242 vs. 28.740 kg; P=0.5271), ADG (0.526 vs. 0.526 kg; P=0.9864), average daily feed intake (0.772 vs. 0.783 kg; P=0.6524), or FCR (1.588 vs. 1.614; P=0.6491). At the end of the trial, blood samples were collected for hematological and biochemical analyses. No differences between treatments were observed in leukogram, erythrogram, or serum biochemical parameters. In conclusion, the inclusion of BSF larvae oil as a substitute for soybean oil in post-weaning piglet diets can be implemented without adverse effects on growth performance. Future studies should include a larger sample size and evaluate different inclusion levels.

### Effect of the application of a phytogetic complex and a probiotic on the kinetic indicators of the semen of rams (Mutton Charollais) outside the breeding season

N. Miteva<sup>1</sup>

<sup>1</sup> Academy of Agriculture, Institute of Agriculture, Stara Zagora, 6000 Stara Zagora, Bulgaria

Effect of the application of a phytogetic complex and a probiotic on the kinetic indicators of the semen of rams (Mutton Charollais) outside the breeding season Natalya Miteva\*, Nikolay Ivanov, Ivan Slavov, Kamelia Zhelyazkova, Ivelina Aleksandrova, Stayka Laleva Academy of Agriculture, Institute of Agriculture – Stara Zagora, Bulgaria \* Corresponding author: nataliya.z.miteva@abv.bg Abstract The objective of the present study is to monitor the effect of the combination of biologically active substances contained in three plant species (thyme – *Thymus vulgaris* L., oregano – *Origanum vulgare* L. and tackweed – *Tribulus terrestris*) and probiotic – ZOOVIT LL, on the kinetic indicators of the semen of rams of the Mouton Charollais breed outside the breeding season. The experiment was conducted at the Agricultural Institute – Stara Zagora (March–April 2025). The subjects of the study were 18 clinically healthy rams divided into three groups (control and two experimental). The analysis of the ejaculates was performed using a computer-assisted system (CASA) with AndroVision® software. The results showed that the supplement had a statistically significant modulation on key indicators of the sperm profile, including total and progressive motility, as well as the levels of slow-moving, circular (CM) and dead (IM) spermatozoa. Although no significant differences were observed in the initial stage (0–21 days), in the final phases of the study, an improvement in cell kinematics was observed in the treated groups compared to the control one. The data obtained confirmed the potential of plant bioactive substances and probiotics for optimizing the reproductive capacity in sheep outside the breeding season. The results contribute to the development of sustainable nutritional strategies for improving reproductive efficiency in meat sheep farming. Keywords: semen, kinetic indicators, Mouton Charollais, *Tribulus terrestris*, *Thymus vulgaris* L., *Origanum vulgare* L., probiotic, rams

### Characteristics of yogurt made from milk from goats fed with white grape pomace silage

T. Romero<sup>1</sup>, M. C. Beltrán<sup>1</sup>, J. Carpintero<sup>1</sup>, M. Gálvez-López<sup>2</sup>, R. Montes<sup>3</sup>, M. P. Molina<sup>1</sup>

<sup>1</sup> Institute of Animal Science and Technology, Universitat Politècnica de València, Camino de Vera s/n., 46022 València, Spain, <sup>2</sup> Research and Innovation Centre for Agri-food and Agro-Environmental Technologies, Miguel Hernández University of Elche, Benier Road, km 3.2, 03312 Orihuela, Spain, <sup>3</sup> Applied Microbiology Advanced Research Centre, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

The aim of this study was to evaluate the effect of including white grape pomace silage (WGP) in the diet of dairy goats on the quality characteristics of yogurt. Two groups (n= 20) of Murciano-Granadina goats from the ICTA experimental herd were used, one receiving a control diet (C) and the other a diet supplemented with 15% WGP. Three batches of yogurt were produced using pasteurised milk and a commercial starting culture. The yogurt's physicochemical and microbiological properties were analysed after 1, 14 and 28 days of cold storage. A sensory evaluation of yogurt was also carried out after 14 days of cold storage. A multifactorial ANOVA was applied to evaluate the effect of the diet and storage time on all parameters studied and the differences in sensory analysis were estimated using Student's t-test. Results showed that the diet had no effect on the manufacturing process, although it did affect the chemical composition and colour parameters. The WGP yogurts presented higher fat (p<0.05) and lower protein content (p<0.05), while all colour parameters decreased (L and coordinates a\* p<0.05, and b\* p<0.01) compared to C group. Regarding yogurt storage, the pH (p<0.05), lactose levels and lightness (L) reduced over time (p<0.01), while °Dornic values increased (p<0.01). As for microbial population, the average CFU/g was unaffected (p>0.05) by the storage period. The sensory analysis results showed no differences in any attribute evaluated (p>0.05). The diet per storage time (D x S) interaction was not significant (p>0.05) in the study. These results suggest that incorporating WGP in goat diets could be beneficial, as it did not negatively affect yogurt production and quality. Additionally, it may help reduce feed costs and optimise waste management in the winery industry. Keywords: yogurt, goat, white grape pomace, agro-industrial waste

**Effect of the inclusion of white grape pomace into the diet of dairy goats on the antioxidant capacity and lipid profile of ripened Tronchón type cheese**

T. Romero<sup>1</sup>, M. C. Beltrán<sup>1</sup>, A. Martínez<sup>1</sup>, M. P. Molina<sup>1</sup>, M. Rodríguez<sup>1</sup>, C. Peris<sup>1</sup>

<sup>1</sup> Institute for Animal Science and Technology (ICTA), Universitat Politècnica de València, Camino de Vera, s/n, 46022 Valencia, Spain

The effect of white grape pomace (WGP) inclusion in dairy goats' diet on quality characteristics of ripened Tronchón cheeses was evaluated. Two homogeneous groups of Murciano-Granadina goats (n= 17 each) belonging to the experimental herd of the ICTA were used, each fed with different diets: a standard diet (Control) and an experimental diet containing 15 % of WGP silage, on a dry basis, replacing part of the alfalfa hay (WGP). Three independent batches of ripened cheeses were produced at a pilot-scale using milk (50 litres) from each experimental group. Cheeses were analysed (two cheeses/batch) at different ripening times (1, 30 and 60 days) for gross composition, antioxidant capacity and lipid profile. A multifactorial ANOVA was applied to evaluate the effect of the diet (Control vs WGP), cheese-making trial (1, 2 and 3) and ripening time (1, 30 and 60 days) on the chemical composition and functional parameters of the Tronchón cheeses. Results indicated that WGP diet did not produce significant improvements in gross composition or antioxidant capacity of the cheeses, which were similar ( $p > 0.05$ ) in both dietary treatments. However, positive changes were observed in the lipid profile of the cheeses, with a reduction in the saturated fatty acids content (70.96 % vs 71.74 % for WGP and Control group cheeses, respectively;  $p < 0.05$ ), and in the cardiovascular risk indices calculated (atherogenic: 2.44 vs 2.61;  $p < 0.01$  and thrombogenic: 1.77 vs 2.02;  $p < 0.001$ ), as well as an increase in monounsaturated fatty acids (23.33 % vs 22.7 %;  $p < 0.05$ ), polyunsaturated fatty acids (6.23 % vs 5.85 %;  $p < 0.05$ ) and conjugated linoleic acid (CLA: 1.41 vs 1.26;  $p < 0.01$ ). Ripening time was the factor that most influenced the functional parameters studied, with an increase in the antioxidant capacity of the cheeses ( $p < 0.001$ ) as ripening progressed, regardless of the type of diet (diet  $\times$  ripening time interaction,  $p > 0.05$ ). These outcomes support the use of grape pomace as a viable agro-industrial resource from both a nutritional and environmental perspective, promoting its incorporation as a sustainable strategy in ruminant feeding without compromising the final product quality. Keywords: goat milk, goat cheese, white grape pomace

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**effects of graded levels of *Mormodica balsamina* L. (Balsam apple) inclusion in complete diets on pre-weaning performance and nutrient utilization of Uda ewes and their lambs**

K. M. Aljameel<sup>1</sup>, B. M. Musa<sup>1</sup>

<sup>1</sup> Usmanu Danfodiyo University, Sokoto, Animal Science, Sokoto, 234 Sokoto, Nigeria

This study evaluated the effects of graded levels of *Mormodica balsamina* L. (Balsam apple) inclusion in complete diets on pre-weaning performance, milk yield, milk composition, and nutrient utilization of Uda ewes and their lambs. The study was conducted at the Usmanu Danfodiyo University Livestock Teaching and Research Farm. The Farm is located within the Main Campus of the University at about 10km North of Sokoto Metropolis in Wamakko Local Government Area of Sokoto State. Sixteen first-parity Uda ewes and four rams were randomly assigned to four dietary treatments containing 0%, 2.5%, 5%, and 7.5% M. balsamina. Animals were fed ad libitum for 24 weeks. Live weight, feed intake, milk yield, milk composition, and pre-weaning lamb growth were recorded. The milk yield was estimated by measuring the weight gain of the lambs weekly. Data on daily voluntary and nutrient intakes, average daily gain (ADG), initial and final live- weights, nutrient digestibility coefficients, nitrogen retention and milk composition was analyzed using analysis of variance (ANOVA). Treatment means were separated using Duncan Multiple Range Test. Results indicated no significant differences ( $P > 0.05$ ) in birth weight and weaning weight among lambs, but weight gain (6.91–8.55 kg), average daily gain (82.26–101.79 g/day), and milk yield (398.55–453.25 ml/day) varied significantly ( $P < 0.05$ ), with higher values observed at 2.5% and 7.5% inclusion levels. Milk chemical composition showed no significant differences in fat, protein, total solids, and lactose, while ash, density, pH, solid-not-fat, and acidity varied significantly ( $P < 0.05$ ). Mineral content analysis revealed significant differences in K, Na, Zn, and P, while Mg, Ca, Fe, Mn, and Cu were not significantly affected. The enhanced milk yield and improved pre-weaning growth suggest that M. balsamina can positively influence lactation performance and nutrient utilization in ewes, translating into better growth of lambs. Inclusion of M. balsamina up to 7.5% in the diet is therefore safe, does not adversely affect milk quality, and supports optimal lamb growth.

**Mitigating Aflatoxin B1 Hepatotoxicity: The Role of Herbal-Mediated miRNA Regulation in Pigs**

G. Kalra<sup>1</sup>, S. Sultana<sup>1</sup>, N. Purohit<sup>1</sup>, V. K. Asediya<sup>1</sup>, C. S. Pareek<sup>1</sup>, P. Sharma<sup>2</sup>, K. Kibitlewska<sup>2</sup>, A. A. Nalpadan<sup>3</sup>, K. Wimmers<sup>3</sup>  
<sup>1</sup> Nicolaus Copernicus University, Department of Infectious, Invasive Diseases and Veterinary Administration, Faculty of Biological and Veterinary Sciences, Centre for Modern Interdisciplinary Technologies, 87-100 Torun, Poland, <sup>2</sup> University of Warmia and Mazury, Department of Pig Breeding, Department of Animal Biochemistry and Biotechnology, Faculty of Animal BioEngineering, 10-719 Olsztyn, Poland, <sup>3</sup> Research Institute for Farm Animal Biology, FBN, 18-196 Dummerstorf, Germany

AflatoxinB1, a potent hepatotoxin, disrupts liver function by interfering RNA regulation. While medicinal herbs like *Andrographis paniculata* & *Silybum marianum* are recognized for their hepatoprotective properties, specifically the role of miRNA modulation—remain poorly defined. A feeding experiment was conducted using 154 pigs. 3 cohorts received standardized diets supplemented with AP (30 mg/kg BW), SM & CL (90mg/kg BW) while a control group (N=24) with AFB1 only. RNA libraries were sequenced and analyzed using differential expression of genes (DEGs) and weighted gene co-expression network analysis (WGCNA). Preliminary data indicate that both AP and SM supplementation significantly modulated miRNA expression profiles associated with hepatoprotection. These changes were reflected in (DEGs) (P<0.05) involved in critical immune response and detoxification pathways. Notably, common miRNA signatures were identified across all herbal treatments viz., all herbs (N=25), AP&SM (N=14), AP&CL (N=5), SM&CL (N=2) suggesting shared regulatory mechanisms. In contrast, Curcuma supplementation did not result in significant transcriptomic changes in porcine liver miRNA expression. Ongoing WGCNA and KEGG pathway enrichment analyses aim to further elucidate the specific molecular pathways underlying these protective effects. These findings suggest that AP and SM exert substantial hepatoprotective effects against AFB1-induced toxicity through miRNA-mediated regulation of immune and metabolic pathways. The identification of shared miRNA signatures highlights potential universal molecular targets for herbal-mediated liver protection in swine. This research is financed and supported by the scientific project NCN-OPUSLAP (UMO-2021/43/I/NZ9/02612) and (DFG)—Project number 504983453 (WI 1754/18)

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**Effect of supplying individual doses of concentrates according to milk yield in mid-lactation dairy sheep**

M. A. Porcu<sup>1</sup>, F. Castangia<sup>1</sup>, A. Ledda<sup>1</sup>, A. Cannas<sup>1</sup>

<sup>1</sup> Department of Agricultural Sciences, Viale Italia 39/A, 07100 Sassari, Italy

One of the main challenges in dairy sheep farms in Sardinia is that all ewes within a flock are typically fed the same ration, thus forming a single feeding group. This strategy is considered a possible cause of low milk yield, reduced lactation persistence, and increased feed wastage, even though on this issue the literature on sheep is scanty. Therefore, this study investigated the effects of individually supplying concentrate doses based on milk yield in dairy sheep. Trial was carried out from May 14 to June 16 2025 (34 days) and the experimental period was divided into two subperiods (P1 and P2). During P1 the ewes had access to ryegrass pasture for 9 hours daily, while in the P2 they were housed and fed exclusively indoors. The trial involved 22 Sarda ewes in their third month of lactation, divided into two groups: control (CNT) and treatment (TRT). The CNT group received an individual fixed dose of whole corn grains (600 g/day per ewe as fed), whereas the ewes of TRT group were fed individually specific doses of corn grains (ranging from 420 to 920 g/day per ewe as fed, with a group average of 600 g/day) based on each ewe's nutritional requirements. Data on intake, milk yield and composition and estimated energy balance (eEB) were analyzed using ANCOVA. Estimated total DMI was significantly higher in TRT than in CON (P<0.001) during P1, due to a higher daily TMR intake (P = 0.001), but was not affected by the treatment during P2. The results of this study showed that individual corn grain supplementation based on milk yield during mid-lactation did not significantly affect milk production and composition in either experimental subperiod. The eEB was significantly higher in CON than TRT during P1, with values of 1.39 and 1.13 Mcal/d, respectively (P<0.001). In P2, eEB did not differ between groups, with values of 1.60 and 1.45 Mcal/d for CON and TRT, respectively. However, an increasing trend in eEB was observed in both groups from the pre-experimental period to P2 as lactation advanced. In conclusion, the supply of whole corn grains in individual doses according to milk yield during mid-lactation may have contributed to an improvement in EB without improving the milk yield.

### Optimisation of rabbit feeding: effects of rapeseed-free diet on lipid profile, oxidative stability and sensory quality of meat

C. Vieira Aller<sup>2</sup>, A. Benito Diaz<sup>1</sup>, B. Martínez Domínguez<sup>2</sup>, R. Bodas Rodríguez<sup>1</sup>, A. Sarmiento García<sup>3</sup>, J. J. García García<sup>1</sup>

<sup>1</sup> Technological Agricultural Institute of Castile and León (ITACyL), Ruminant Research Unit, Burgos-Portugal road, km. 119, 47071 Valladolid, Spain, <sup>2</sup> Technological Agricultural Institute of Castile and León (ITACyL), Technological Station of Meat, Filiberto Villalobos Street, 5, 37770 Guijuelo, Spain, <sup>3</sup> University of León, Department of Animal Production, Vegazana Campus, s/n. Faculty of Veterinary Medicine, 24007 León, Spain

Rabbit meat is characterised by a low-fat content and a high protein value; however, its sensory acceptance may be influenced by the composition and stability of its lipid fraction. Ingredients such as rapeseed have been associated with changes in meat flavour and oxidative stability, prompting interest in alternative formulations. To evaluate the specific effect of rapeseed removal, a feeding trial was conducted comparing a standard control diet (CON) with a rapeseed-free diet (RF). Both diets were formulated to be isoproteic and isoenergetic. Twenty rabbits were included in the study, with 10 animals allocated to each dietary group. Dietary treatments were applied for 40 days, and animals were slaughtered at 75 days of age. Samples were collected from perirenal fat and the shoulder, as well as from the loin and fore and hind limbs. Rapeseed removal did not affect carcass weight or total fat percentage. However, rabbits fed the RF diet showed a higher  $\omega$ -3 polyunsaturated fatty acid content and a lower  $\omega$ -6/ $\omega$ -3 ratio ( $p < 0.05$ ). These changes were accompanied by reduced oxidative stability, evidenced by increased concentrations of oxidation-derived aldehydes ( $p < 0.05$ ), and were associated with rancid sensory notes and lower sensory acceptability. Overall, the results indicate that eliminating rapeseed from isoproteic and isoenergetic diets modifies the lipid profile of rabbit meat but may increase oxidative susceptibility, highlighting the need to balance nutritional improvement with technological and sensory quality when designing feeding strategies.

### Dietary Olive Pomace and Reproductive Performance of Majorcan Black Pigs

J. Cladera<sup>1,2</sup>, M. Llompard<sup>2</sup>, I. Sastre-Conde<sup>3</sup>, P. A. Company<sup>1</sup>, J. Jaume<sup>3</sup>

<sup>1</sup> Can Company, C. Sastre, 9 local 12, 07300 Inca, Spain, <sup>2</sup> Universitat de les Illes Balears, Ctra. de Valldemosa, 7,5, 07122 Palma, Spain, <sup>3</sup> Institut de Recerca i Formació Agroalimentària i Pesquera de les Illes Balears, C. d'Eusebi Estada, 145, 07009 Palma, Spain

The Majorcan Black Pig (MBP) is a native breed of Majorca with its own extensive production system. In terms of production, it has much lower yields than current intensive pig production systems, as do other extensive Mediterranean systems. It also has a high capacity for fat accumulation. Moreover, low birth weight can be attributed to the high proportion of piglets born weighing less than one kilogram, leading to increased mortality, stunted growth and higher production costs. In this context, using products derived from olive oil production, such as olive pomace (known as 'alperujo', AL), in animal feed could be an alternative in sustainable livestock production systems, helping to mitigate environmental issues arising from its treatment as waste. AL is a semi-solid organic by-product of oil production comprising pulp, skins, pits and vegetable water from olives, characterized by a high fibre, fatty acid, and bioactive compound content. The objective of this study was therefore to evaluate the effects of AL in the nutrition of pregnant MBP sows on reproductive performance. Eighteen newly weaned MBP sows, matched for previous production results, backfat thickness and age, were randomly allocated to two treatments ( $n = 9$  per group): a control group fed cereals and legumes and an experimental group receiving the same diet with free access to AL from weaning to farrowing (January–June). Data were analysed using one-way ANOVA and Duncan's multiple range test ( $p \leq 0.05$ ). The experimental results showed that voluntary consumption of AL amounted to  $0.2 \text{ kg day}^{-1}$ , and MBP sows receiving AL during gestation had almost one more piglet per litter, fewer low-weight piglets and higher average piglet weight, while the remaining reproductive parameters and backfat thickness were not significantly affected. These results are encouraging from a production perspective, as AL can be considered a valuable ingredient in MBP sow diets, contributing to improved reproductive efficiency and sustainability within a circular economy framework.

### Ensiled and dehydrated grape pomace in the production of lamb meat

M. Ponte<sup>1</sup>, S. Faryadi<sup>1</sup>, R. Badalamenti<sup>1</sup>, A. Bonanno<sup>1</sup>, A. Di Grigoli<sup>1</sup>

<sup>1</sup> Università degli Studi di Palermo, Dipartimento Science Agrarie, Alimentari e Forestali, Viale delle Scienze, 90128 Palermo, Italy

Among agri-food by-products widely available in Italy, grape pomace (GP) receives growing interest as feeding resource in sustainable livestock, especially due to the antioxidant activity of its polyphenols content. However, due to its high moisture, the stabilization of GP is necessary to entirely exploit its potential in animals' feeding. Thus, two different conservation methods, such as ensiling and dehydration, have been compared based on the effects on lamb meat. Two experiments (EXP) were carried out to evaluate the effects of GP, stabilized by ensiling (EGP) or dehydration (DGP) and included in the diets of nursing ewes (EXP1) or male lambs after weaning (EXP2), on growth performance and meat quality of suckling lambs (n. 24) and fattening lambs (n.24). Both nursing ewes and weaned lambs were divided into three homogeneous groups fed a control diet (CON) consisting of hay and concentrate feed, or the same diet integrated with EGP (1.2 and 1.0 kg/d to each ewe or lamb) or GPD (0.6 and 0.5 kg/d to each ewe or lamb). The EXP1 lasted 6 weeks, from lambing to slaughter, during which lambs suckled milk as only feed; whereas in EXP2 the fattening period lasted 9 weeks, from weaning (45 d) to slaughter (110 d). In EXP1, no difference emerged for growth rate and carcass and meat traits of suckling lambs, except for polyphenols content and antioxidant capacity of meat, being higher in EGP than in CON group; moreover, the level of rumenic acid (RA) of meat fat increased with both GP-based diets. In EXP2, EGP lambs showed higher growth rate and live weight at slaughter than the other groups; the meat of lambs fed GP was characterized by higher polyphenols content, greater antioxidant capacity, and better fatty acid profile due to higher RA content and more favorable health promoting index. Ultimately, the use of GP did not negatively affect the lambs' growth performance. Whereas ensiling proved to be a profitable method to stabilize GP, in both economic and functional terms; indeed, EGP were able to preserve the polyphenols content and transfer their bioactive properties to meat of both suckling and fattening lambs. Research supported by PRIN 2022 grant – 20227BXEYZ – ByByWaste

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### Effects of additives supplementation on milk production and health parameters in Sarda dairy ewes under heat stress

A. S. Atzori<sup>1</sup>, M. Contena<sup>2</sup>, F. Castangia<sup>2</sup>, L. Nieddu<sup>2</sup>, A. Ledda<sup>2</sup>

<sup>1</sup> Gi-ANTLab Guided Innovation by Animal New Tech, SpinOff UNISS, P.Crispo 3, 07100 Sassari, Italy, <sup>2</sup> University of Sassari, Agricultural Sciences, Viale Italia 39, 07100 Sassari, Italy

During heat stress (HS), the animal is not able to dissipate the excess of heat load due to the high ambient temperatures. To mitigate the effects of HS, several management strategies can be adopted; among these, the most commonly applied involve the use of dietary additives aimed at reducing metabolic heat production associated with ruminal fibre digestion. A total of 40 Sarda sheep were selected divided in 2 groups homogeneous for milk production, body weight, and Body Condition Score (BCS): control group (CON) and additive group (ADD), that received a commercial additive ThermoTech® based on capsaicin at the dosis of 5 g/d. The following traits were recorded: milk yield, fat and protein corrected milk (FPCM), body weight, BCS, dry matter intake (DMI), and ruminal parameters. Rectal temperature and breath frequency were detected on 12 ewes per group. All the traits were analyzed separately for the pre-experimental and experimental phases using a generalized linear model (GLM) that considered the fixed effects of the time, diet, and their interaction. Means were declared statistically significant for  $P < 0.05$ . During experimental period, body weight and BCS did not differ between the two groups. The DMI was 100 g in ADD vs CON ( $P < 0.001$ ). Milk yield did not differ between the two groups during pre-experimental period, whereas FPCM was significantly higher in ADD vs. CON group during the experimental period (1.114 vs 1.0351 kg/d;  $P = 0.02$ , respectively). Rectal temperature did not differ between the two groups in both phases; breath frequency was higher in CON vs ADD (82.6 vs 78.1;  $P = 0.01$ , respectively). Ruminal parameters did not differ between the two diets. In conclusion, ThermoPlus significantly increased DMI, FPCM, and lowered respiratory rates in the experimental period. Further studies are needed to compare effects of different doses and effects under heat waves in early spring with higher production levels.

### Long-term storage effects on physico-chemical characteristics, fermentation profile, microbiological quality and pathogen presence in baled silage

L. Nieddu<sup>1,3</sup>, M. Fresu<sup>3</sup>, C. Mastroeni<sup>2</sup>, A. Gallo<sup>2</sup>, S. Zara<sup>3</sup>, F. Fancello<sup>3</sup>, A. S. Atzori<sup>3</sup>

<sup>1</sup> IUSS PAVIA, Scuola Universitaria Superiore, Piazza della Vittoria 15, 27100 Pavia, Italy, <sup>2</sup> Università Cattolica del Sacro Cuore, DIANA, Via Emilia Parmense 84, 29122 Piacenza, Italy, <sup>3</sup> Università di Sassari, Dipartimento di Agraria, Viale Italia 39, 07100 Sassari, Italy

The aim of the study was to evaluate the physico-chemical, fermentative and microbiological quality of baled silage samples collected from different farms across Sardinia (n=115). Silage samples were analyzed at 30, 60, 90, 180, and 270 days after baling, with the evaluation of chemical composition, fermentative compounds, the monitoring of five microbial groups both positively and negatively associated with silage quality, and the detection of *Listeria monocytogenes* and *E. coli*. Ensiling length did not affect chemical parameters. The silage showed low NDF content ( $427.9 \pm 76.0$  g/kg DM), with only 7.5% of samples exceeding 50%, and a high average CP content ( $120.4 \pm 32.7$  g/kg DM). Low concentrations of ammonia nitrogen ( $33.56 \pm 27.33$  g/kg TN) and butyric acid ( $2.30 \pm 13.66$  g/kg DM) were observed. Average lactic and acetic acid contents were 3.5% and 1.5% of dry matter, respectively. Their concentrations varied depending on the sampling period, with lactic acid decreasing over time and acetic acid exhibiting an increasing trend. The most important effects were observed on microbiological groups. Lactic acid bacteria (LAB) were found in optimal concentrations ( $5.42 \pm 2.50$  log<sub>10</sub> cfu/g), contributing to a stable average pH of  $4.28 \pm 0.48$ . 73% of the samples were free of Clostridia, and when detected, they were present at low levels ( $1.69$ – $3.46$  log<sub>10</sub> cfu/g). Mold and yeast contamination occurred in 22% of samples ( $2$ – $6.19$  log<sub>10</sub> cfu/g). Neither *E. coli* nor *L. monocytogenes* were detected, indicating correct application of silage production techniques. In conclusion, this study demonstrated that good chemical-fermentative quality is associated with low contamination risks and microbiological safety. These results, rather than referring solely to individual samples, emphasize the crucial importance of a properly managed baling process, which represents the fundamental basis for ensuring the quality and safety of the entire production.

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### Almond hulls in lamb diets: effects on the physical and sensory quality of meat

J. M. Almeida<sup>1,2</sup>, C. Romão<sup>1</sup>, M. R. Marques<sup>1,3</sup>, K. Paulos<sup>1,3</sup>, C. Mestre<sup>1,4</sup>, O. Guerreiro<sup>5,6</sup>, A. T. Belo<sup>1</sup>, E. Jerónimo<sup>5,6</sup>

<sup>1</sup> INIAV, Santarém, Fonte Boa, 2005-048 Vale de Santarém, Portugal, <sup>2</sup> CIISA, FMV-Lisboa, ., 1300-477 Lisboa, Portugal, <sup>3</sup> Geobiotech, FCT Nova, ., 2829-516 Lisboa, Portugal, <sup>4</sup> LEAF, ISA, ., 1300-477 Lisboa, Portugal, <sup>5</sup> CEBAL, ., 7801-908 Beja, Portugal, <sup>6</sup> MED, CHANGE, ., 7801-908 Beja, Portugal

Almond hulls (AH), the primary by-product of almond processing for human consumption, represent a valuable feed resource for ruminants owing to their appreciable energy and fibre content, along with moderate levels of protein. The present study assessed the impact of partial substitution of dehydrated lucerne by almond hulls on meat quality. Thirty-two Merino Branco breed lambs (BW  $19.8 \pm 1.73$  kg) were distributed across four diets containing 0%, 10%, 15%, or 20% dehydrated almond hulls in partial substitution for dehydrated lucerne, within a 60:40 concentrate:forage ratio. After 15 days of adaptation, the 29-day feeding trial was conducted under ad libitum conditions. After slaughter, longissimus dorsi muscle was sampled to evaluate changes in colour, texture, and sensory attributes. Inclusion of dehydrated almond hulls in the diets did not affect colour parameters of either subcutaneous fat or the LD muscle (60 min, 3 and 7 days pm). However, supplementation with AH significantly increase meat shear force at the 10% and 20% inclusion levels (41.2 N and 42.2 N, respectively), whereas the 15% level (36.0 N) did not differ from the control diet (36.0 N). No sensory attribute showed significant differences attributable to the level of dehydrated almond hulls inclusion in the diets. In conclusion, inclusion of up to 20% dehydrated almond hulls in the finishing diets of light lambs can be implemented without compromising meat quality. This work is funded by the project “CAPOTA CIRCULAR FEED – Pilot project on the use of almond hulls in animal feed” (PL24 – 00051), supported by the Promove Program – The Future of the Interior of “la Caixa” Foundation, in collaboration with BPI and Foundation for Science and Technology (FCT). The authors acknowledge the following R&D units: MED (<https://doi.org/10.54499/UID/05183/2025>); CIISA (<https://doi.org/10.54499/UID/00276/2025>); and the Associate Laboratory CHANGE (<https://doi.org/10.54499/LA/P/0121/2020>).

### Valorisation of *Posidonia oceanica* banquettes as a feed ingredient for cattle: an in vitro evaluation of rumen degradability, gas production and methane emission

G. Attard<sup>1</sup>, V. Lopreiato<sup>2</sup>, L. Liotta<sup>2</sup>, A. Minuti<sup>3</sup>, A. Amato<sup>2</sup>, M. Sfulcini<sup>3</sup>, M. Lunetta<sup>2</sup>, T. Meli<sup>1</sup>, E. D'Alessandro<sup>2</sup>

<sup>1</sup> University of Malta, Inst. of Earth Systems, MSD 2080 Msida, Malta, <sup>2</sup> University of Messina, Viale Palatucci, 98168 Messina, Italy, <sup>3</sup> Università Cattolica Sacro Cuore, Via Emilia Parmense, 29122 Piacenza, Italy

The accumulation of *Posidonia oceanica* banquettes on Mediterranean coasts represents an environmental and management issue, highlighting the need for sustainable valorisation strategies. This study evaluated the potential use of *Posidonia oceanica* banquettes as a feed ingredient for cattle through in vitro assays to assess rumen degradability, gas production and methane emission. Four complete low-fermentability diets commonly used for dry cows (LOW) were formulated by progressively replacing straw with *Posidonia oceanica* at 0, 33, 66 and 99% substitution (LOW0, LOW33, LOW66 and LOW99), corresponding to inclusion levels of 0, 10.8, 21.6 and 32.4% on a dry matter basis. Substrates were chemically characterised and incubated in vitro using ruminal fluid from cannulated cows under anaerobic conditions for 24 and 96 h. Measurements included final pH, in vitro organic matter (OM) and neutral detergent fibre (NDF) degradability, total gas production, methane and carbon dioxide production, and fermentation kinetics. Data were analysed using PROC GLM of SAS. After 24 h, final pH was stable across treatments (6.53–6.58). In vitro OM degradability showed minor variation (70.1–71.4%), whereas NDF degradability decreased from LOW0 to LOW99 (38.9 vs. 24.6%). Notably, methane production decreased linearly with increasing *Posidonia oceanica* inclusion, from 9.73 mg/g OM in LOW0 to 8.27 mg/g OM in LOW99. After 96 h, OM degradability declined from 80.8% in LOW0 to 71.1% in LOW99, together with reduced NDF degradability and potential gas production (247 vs. 203 mL/g OM). Overall, *Posidonia oceanica* inclusion reduced methane production and decreased fibre and organic matter degradability, potentially limiting its use in dry cow diets. In addition, the high mineral and salt content of *Posidonia oceanica* should be carefully considered when evaluating its practical dietary inclusion. Despite these constraints, mitigation of methane highlights its potential use to reduce enteric methane emissions.

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### Inclusion of sainfoin in intensive beef cattle diets: implications for growth performance and nitrogen use efficiency

D. Villalba<sup>1</sup>, E. Molina<sup>1</sup>, I. Casasús<sup>2</sup>, M. Joy<sup>2</sup>, M. Blanco<sup>2</sup>

<sup>1</sup> University of Lleida, Animal Science, Av. Rovira Roure 191, 25198 Lleida, Spain, <sup>2</sup> CITA - IA2, Av. Montaña-na 930, 50059 Zaragoza, Spain

The use of locally grown forage legumes can enhance feed self-sufficiency reducing reliance on imported protein sources. Sainfoin (*Onobrychis viciifolia*; SF) can be incorporated into diets of intensively fed cattle either as forage or as an ingredient in concentrate formulations. Forty Montbéliarde young bulls (117 d of age; 188 kg body weight (BW)) were fed ad libitum for 28 weeks with four diets combining two forages (SF hay or barley straw) and two isoenergetic concentrates: a commercial concentrate (C) or a concentrate including 15% SF pellets (CSF). The resulting diets were C+straw, CSF+straw, C+SFhay, and CSF+SFhay. Daily individual BW and concentrate intake were recorded using automated scales and feeders, while forage intake was estimated using an internal marker. Nitrogen (N) balance was assessed at the individual level during two sampling periods using an external marker to estimate faecal output. Also, samples were obtained to analyse ammonia in the ruminal liquid and plasma metabolites (urea and total protein). At the end of the growing phase (week 15), bulls fed C+straw were heavier than those fed C+SFhay, with intermediate values for the remaining diets. By the end of finishing (week 28), bulls fed C+straw remained the heaviest, whereas CSF+straw bulls were the lightest. During the growing period, average daily gain declined with increasing sainfoin inclusion, but concentrate conversion was improved in bulls fed C+SFhay compared with CSF bulls regardless the forage. During finishing, bulls fed CSF+straw showed the poorest feed conversion. During the growing period, CSF+SFhay and CSF+straw reduced urinary N excretion and had lower retained N relative to N intake, indicating improved N use efficiency, especially when related to the different growth of these treatments. Regarding ammonia and urea in plasma, both groups fed CSF had lower values at the growing and finishing periods. Although some sainfoin-based diets resulted in slightly lower growth performance, these effects may be compensated by the adoption of locally based, more circular feeding strategies.

### Influence of rearing system and geographic origin on gut microbiota diversity in *Tenebrio molitor*

Á. Ramos-Martínez<sup>1</sup>, J. Galián<sup>2</sup>

<sup>1</sup> Universidad Pontificia de Comillas, Cam. Viejo Simancas, 47008 Valladolid, Spain, <sup>2</sup> Universidad de Murcia, C. Campus Universitario, 30100 Murcia, Spain

The gut microbiota plays a central role in the health and productivity of insects reared for food and feed. This study evaluated the influence of geographic origin, rearing system, and diet on the gut bacterial community composition of *Tenebrio molitor*. Seven populations representing industrial, traditional, and wild production systems across different geographic regions were analysed. Five larvae per population were processed individually (n = 35), enabling assessment of intrapopulation variability and production system effects. Bacterial communities were characterised using 16S rRNA gene sequencing. Microbial diversity was assessed through alpha diversity metrics (taxonomic richness and Shannon index) and beta diversity analyses based on principal component analysis of relative abundance matrices. Rearing system, diet type, and geographic origin were considered as explanatory variables structuring community patterns. Clear differences in microbial diversity and community composition were observed among populations. Larvae reared under traditional or less controlled systems exhibited higher alpha diversity and more complex community structures, whereas intensively reared populations showed reduced diversity and microbiotas dominated by a limited number of bacterial taxa. Beta diversity analyses indicated greater interindividual variability in manually reared populations compared to intensively reared ones, suggesting that standardised diets and controlled hygienic conditions contribute to microbial homogenisation. Diet composition and substrate moisture emerged as major factors associated with gut microbiota structure, with heterogeneous and fermentable substrates linked to increased bacterial diversity. These compositional differences may influence digestive processes, immune modulation, and resilience to environmental stress under massrearing conditions. Overall, these findings highlight the relevance of microbiota-informed management strategies for optimising *T. molitor* production systems. Adjusting diet formulation and rearing conditions to support a balanced and functionally diverse gut microbiota may contribute to improved stability and sustainability in insect farming.

### Effects of sorghum and extruded sorghum as complete maize substitutes on growth performance and intestinal health of post-weaning piglets

P. Trevisi<sup>1</sup>, F. Palumbo<sup>1</sup>, L. Laghi<sup>1</sup>, A. Zurrú<sup>1</sup>, F. Correa<sup>1</sup>, D. Luise<sup>1</sup>

<sup>1</sup> University of Bologna, Department of Agricultural and Food Sciences, Viale G. Fanin 46, 40127 Bologna, Italy

This study evaluated the effects of completely replacing maize with sorghum on growth performance and intestinal health of post-weaning pigs using an integrated multi-omics approach. From weaning (d0) to 28 days post-weaning, 522 piglets were assigned to one of three dietary treatments: a maize-based control diet (CTR), a sorghum-based diet (SO), or an extruded sorghum-based diet (EX\_SO). Pigs were fed two-phase diets (d0–6 and d7–28). Body weight, average daily gain, feed intake, feed conversion ratio, body lesions and behavioural measurements were recorded weekly. Faecal samples collected at d7 and d28 were analysed for microbiota composition (16S rRNA gene sequencing), metabolomics (<sup>1</sup>H-NMR), ammonia (NH<sub>3</sub>), and dry matter (DM) content. Dietary treatment did not affect growth performance, feed efficiency, lesion scores, or behavioural measurements. At d7, faecal NH<sub>3</sub> and DM content were not influenced by diet, although differences in microbial taxa and metabolites were observed, with higher abundance of *Oscillospira* in CTR compared with EX\_SO (P<0.01) and increased concentrations of fermentation-related metabolites (2-Oxogutarate, Acetoin, 2-Methyl 3-Ketovalerate, 3-Methyl 2-Oxovalerate) in EX\_SO. At d28, faecal DM tended to be higher in SO compared with CTR (P=0.08), and diet affected beta-diversity (P=0.05). Sorghum inclusion and processing were associated with distinct microbial profiles; *Olsenella* was more abundant in the CTR than in the SO (P<0.01); *Olsenella* (P=0.01) and *Ligilactobacillus* (P=0.01) were more abundant, while *Anaerovoracaceae*\_Family XIII UCG-001 (P=0.01) was less abundant in the CTR group than in the EX\_SO. Butyrate, propionate, and valerate more abundant in the EX\_SO than CTR. In conclusion, sorghum, either non-extruded or extruded, can replace maize in post-weaning pig diet without impairing growth performance, behavioural welfare, or intestinal health, while modulating gut microbial and metabolic profiles. These results support sorghum as a sustainable feed ingredient for pig diet. Acknowledgements SustainableHeavySuis Project (J33C23002980002)–L.R. n. 17/2022-DGR 165 del 06/02/2023, Emilia Romagna Region, Italy.

### Effect of calving season on feeding behavior and body weight dynamics of native suckler cows in Mediterranean natural pastures

S. Khater<sup>1</sup>, G. Battacone<sup>1</sup>, M. Decandia<sup>2</sup>, V. Giovanetti<sup>2</sup>, M. Acciaro<sup>2</sup>

<sup>1</sup> Università degli studi di Sassari, Dipartimento di Agraria, Viale Italia 39/A, 07100 Sassari, Italy, <sup>2</sup> AGRIS Sardegna, Fertilia 291, 07100 Sassari, Italy

In Mediterranean extensive systems, nutritional constraints are strongly shaped by seasonal pasture dynamics, making the alignment between reproductive timing and feed availability a critical management factor. This study, conducted between May and June 2022 in a mountainous region of Sardinia, evaluated the effect of calving season on feeding behavior, herbage intake and body weight recovery of native Sarda suckler cows managed under continuous grazing on natural silvopastoral pastures. Two groups were compared, winter-calving (group W) cows in late lactation and spring-calving cows (group S) in early lactation, both grazing the same pasture without supplementation. Behavior was monitored through focal animal sampling, while herbage dry matter intake (HDMI), digestibility (HDMD) and fecal output (FO) were estimated using the n-alkane technique. The pasture provided 2210 ± 690 kg DM/ha and was dominated by Poaceae (73.4%), followed by Fabaceae (10%) and other families (16.6%); grazed herbage showed moderate protein content (15.8 ± 3.1% DM) and high fiber levels (NDF 53.6 ± 6.1% DM; ADF 29.6 ± 2.2% DM). Group S showed higher grazing time than Group W in the subperiods where group differences were significant and achieved significantly greater HDMI relative to body weight (3.58 ± 0.210 DM/100 kg BW) than group W (2.81 ± 0.208 kg DM/100 kg BW). In contrast, group W showed higher body weight and recovery rate (BW: 478.239 ± 5.9 kg, RR: 0.586 ± 0.058 kg/day) than group S (BW: 432.161 ± 5.915, RR: 0.084 ± 0.058). Ruminating, resting, and walking activities were not significantly different between the two groups, likely reflecting social facilitation effects arising from shared conditions within the herd. Despite the differences in intake and performance, HDMD and FO did not differ between groups, revealing similar forage quality and digestive efficiency. These results demonstrate that lactation stage interacts with seasonal pasture conditions to shape feeding strategies and energy balance. The findings provide quantitative evidence to support nutritional planning in low-input pasture-based systems.

### The dampwood termite *Hodotermopsis sjostedti* as a dietary source for broiler chickens

Y. Tsukahara<sup>1</sup>, N. Tomonaga<sup>1</sup>, E. Tasaki<sup>1,2</sup>, Y. Mitaka<sup>1,3</sup>, S. Tomonaga<sup>1</sup>, H. Kumagai<sup>1</sup>, H. Hirooka<sup>1</sup>, K. Matsuura<sup>1</sup>

<sup>1</sup> Kyoto University, Graduate School of Agriculture, Kitashirakawa-Oiwakecho, Sakyo, 606-8502 Kyoto, Japan, <sup>2</sup> Niigata University, Department of Life and Food Sciences, Graduate School of Science and Technology, Ikarashi 2-no-cho, Nishi-ku, 950-2181 Niigata, Japan, <sup>3</sup> Nagoya University, Graduate School of Bioagricultural Sciences, Furocho, Chikusa, 464-8601 Nagoya, Japan

The use of insects in animal feed has garnered growing attention as a sustainable and alternative nutritional resource. In this study, we evaluated the safety and nutritional profile of the dampwood termite *Hodotermopsis sjostedti*. We also conducted 2 short-term feeding trials using Ross 308 female broiler chickens to assess the potential of termite meal as a novel feed ingredient for future large-scale applications. Colonies were lab-reared, and termite individuals of entire colonies were collected by carefully crushing the wood, freeze-dried, and ground into meal. The resulting termite meal contained 65.6% crude protein, 16.0% fat, and 5.2% ash on a dry matter basis. No minerals were at toxic concentrations. Two feeding experiments using broilers evaluated the effects of termite meal inclusion. In Experiment 1, 20 chickens (8-day-old) were assigned to 4 dietary treatments: a commercial diet plus 5.0% fish meal, and the fish meal was substituted with 0.5%, 2.5%, or 5.0% termite meal. In Experiment 2, 18 chickens (9-day-old) were assigned to 3 diets: control, control + 2.5% termite, or control + 2.5% fish meal. Body weight and feed intake were recorded daily, and samples were collected at 21 days of age. Growth performance, organ weights, and blood plasma profiles were comparable across treatments, with few exceptions. Chickens receiving greater termite meal concentrations had numerically greater alanine aminotransferase, suggesting a potential hepatic burden. No adverse effects on growth and meat and organ weights were observed. In conclusion, *H. sjostedti* meal demonstrates strong potential as a novel dietary component for broiler chickens. However, further long-term studies with an increased number of birds will be necessary to ensure its safe and effective use in poultry diets.

**Breeding for Resilience-GWAS insights from Baladi goats for sustainable parasite control\***

J. Shamaii<sup>1,9</sup>, S. El Murr<sup>2,3</sup>, M. Saad<sup>1</sup>, R. Hindi<sup>4</sup>, E. El Hajj<sup>1,3</sup>, K. Houchaymi<sup>2</sup>, B. Murdoch<sup>5</sup>, J. Burke<sup>6</sup>, K. Jawasreh<sup>7,8</sup>, P. Aad<sup>1</sup>

<sup>1</sup> Notre Dame University, Sciences, -, - Zouk Mosbeh, Lebanon, <sup>2</sup> LARI, Animal Research Station, -, - Terbol, Lebanon, <sup>3</sup> ULFAV, Animal Science, -, - Dekwaneh, Lebanon, <sup>4</sup> USJ, ESIAM, -, - Taanayel, Lebanon, <sup>5</sup> University of IDAHO, Animal, Veterinary and Food Sciences, -, - Moscow, ID, United States, <sup>6</sup> USDA, Dale Bumpers Small Farms Research Center, -, - Booneville, AZ, United States, <sup>7</sup> JUST, Animal Production, -, - Irbid, Jordan, <sup>8</sup> Abu Dhabi Agriculture and Food Safety Authority, Applied Research, -, - Abu Dhabi, United Arab Emirates, <sup>9</sup> Saint Louis Hospital, -, -, - Jounieh, Lebanon

The Lebanese local Balagi goats are well adapted to Mediterranean conditions. Despite facing significant gastrointestinal parasite challenges, their performance and production remain stable and show high resilience under harsh circumstances. This phenomenon underscores an urgent need for identifying possible genetic markers of different types of resilience. For the first time, a genome-wide association study using the 70K Illumina goat SNP chip was performed on 202 Baladi does from managed, pastoral, and agro-pastoral systems, from Lebanon. Fecal egg counts for *Eimeria*, *coccidia* and the most prevalent gastrointestinal nematodes were assessed. FAMACHA anemia score and BCS were recorded, and temperature-humidity index (THI) calculated. GLM procedure of SAS was used to study the fixed effects (management, period, age). Chi-square from PLINK within R and Bonferroni multiple comparison were applied to identify the most statistically significant SNPs associated with resilience. Ten novel loci were significantly associated with resilience traits, one SNP on chromosome 10 linked to heat-stress resilience, loci on chromosomes 9, 19, and 25 were linked to *Eimeria* burden and loci on chromosomes 1, 11, 14, and 15 linked to total nematode burden, all near genes involved in immune function and metabolic adaptation. These loci could constitute genomic targets for marker-assisted selection of parasite-resilient goats and diminish reliance on chemical anthelmintics to foster sustainable Mediterranean goat farming and conserving local genetic resources. \*Supported by PEER-USAID, AID-OAA-A-11-00012.

## Session 7

## Theatre 2

**Physiological traits shaped by family structure and altitude in Lojeña sheep: insights into genetic selection for climate resilience**

C. Iglesias Pastrana<sup>1</sup>, E. Díaz Ruiz<sup>2</sup>, L. Marín Marín<sup>2</sup>, M. Aguilera Ropero<sup>3</sup>, M. E. Camacho Vallejo<sup>1</sup>, J. V. Delgado Bermejo<sup>2</sup>

<sup>1</sup> Andalusian Institute for Agricultural, Fishing, Food and Ecological Production Research and Training (IFAPA), Department of Agriculture and Ecological Husbandry, Centro 'Alameda del Obispo', 14004 Córdoba, Spain, <sup>2</sup> University of Córdoba, Genetics Department, C5 Gregor Mendel building, Rabanales University Campus, 14071 Córdoba, Spain, <sup>3</sup> Asociación de Ganaderos Criadores de la Raza Ovina Lojeña del Poniente Granadino, Avda .R. Pérez del Álamo, s/n, 18300 Loja, Spain

Understanding the sources of variation in physiological traits is relevant for endangered local breeds in extensive systems. This study quantified, using nested mixed-effects models at the individual level, family-related and environmental contributions to physiological, hematological and biochemical traits in Lojeña sheep (n = 583) from 44 family groups. Animals were classified into four altitude-related management groups: group 1, farms below 980 m a.s.l. without grazing (n = 4 animals); group 2, farms below 980 m a.s.l. with grazing up to 1391 m a.s.l. (n = 27 animals); group 3, farms below 980 m a.s.l. with grazing above 1391 m a.s.l. (n = 181 animals); and group 4, farms above 980 m a.s.l. with grazing up to 1391 m a.s.l. (n = 317 animals). Altitude group was considered an environmental factor, with family nested within altitude, allowing the partitioning of variability into altitude-, family- and individual-level components. Traits were mainly interpreted based on the proportion of variance attributed to family within altitude (≥10%). Heart rate, rectal temperature, creatinine, LDH, mean corpuscular hemoglobin concentration, lymphocytes and platelets, showed a relevant family-related component together with relatively low altitude-related variance and therefore represent the most promising candidates for genetic selection aimed at improving climate resilience. In contrast, urea, triglycerides, total proteins and GOT were mainly influenced by altitude and are more likely to respond to management strategies. This approach supports informed decision-making in conservation and breeding programs for local sheep breeds.

### Smart Family Livestock: Integrating AI and Structured Breeding to Enhance Resilience and Productivity of Mediterranean Native Breeds

M. Djemali<sup>1</sup>, A. Hamrouni<sup>1</sup>, F. Ben Khalifa<sup>2</sup>

<sup>1</sup> INAT, Animal Genetic and Feed Research Lab., 43, Avenue Charles Nicole, 1082 Tunis, Tunisia, <sup>2</sup> UTAP, Animal production, Av. Alain Savary, 1003 Tunis, Tunisia

This study proposes a first operational framework of Smart Family Livestock (SFL), a model designed to strengthen the resilience of Mediterranean family livestock systems by positioning the family farm as a structured economic unit embedded within integrated forage–livestock systems and supported by digital tools and collective breeding schemes. The SFL framework is structured around four pillars: (i) family-centered livestock entrepreneurship aimed at stable income generation; (ii) forage–livestock integration to improve resource-use efficiency and climate resilience; (iii) artificial intelligence (AI)–based decision-support tools for feeding, reproduction, animal health, and farm management; and (iv) participation in organized breeding and performance recording systems to enable genetic improvement of native breeds. The framework is illustrated using field-level performance data from an elite Black Thibar sheep flock in Tunisia managed under a nucleus–multiplier breeding structure with approximately 1,000 producing ewes. Growth performance records from 389 lambs, including 178 recorded in 2023 and 211 in 2024, were collected through routine on-farm recording under real Mediterranean production conditions. The analytical approach combines descriptive performance analysis with scenario-based modelling of genetic dissemination under conservative assumptions related to ram production rates and mating capacity in family flocks. Selection of breeding rams was based on recorded growth performance under standard management and controlled mating. Under the current structure, comprising a nucleus of 250 ewes and a multiplier of 750 ewes, the system can produce more than 500 improved rams, sufficient to serve over 15,000 ewes. An expansion scenario involving additional family farmers, with a nucleus of 500 ewes and a multiplier of 1,500 ewes, would allow the annual production of over 1,000 improved rams, benefiting more than 35,000 ewes. These results highlight the potential of SFL to enhance genetic improvement, productivity, and resilience of native-breed-based family farming systems under Mediterranean conditions.

### Session 7

### Theatre 4

#### Identifying “who and what” to mitigate: clustering-based carbon footprint profiles in European dairy sheep farms

M. D. G. Azzena<sup>1</sup>, C. Mussolino<sup>1</sup>, B. Darabighane<sup>1</sup>, O. Del Hierro<sup>2</sup>, C. Buckley<sup>5</sup>, S. Throude<sup>6</sup>, G. Serra<sup>4</sup>, C. Dragomir<sup>3</sup>, A. S. Atzori<sup>1</sup>

<sup>1</sup> Department of Agriculture Section of Animal Science, Viale Italia 39a, 07100 Sassari, Italy, <sup>2</sup> Parque Tecnológico, Parcela 812, 48160 Bizkaia, Spain, <sup>3</sup> Institutul National de Cercetare, on Ionescu de la Brad 6, 013813 Dezvoltare, Romania, <sup>4</sup> AGRIS Sardegna, Loc. Bonassai, 07100 Sassari, Italy, <sup>5</sup> Teagasc, Mellows Campus, H65 R718 Athenry, Ireland, <sup>6</sup> Institut de l'Élevage, ue de Bercy, 75012 Paris, France

Simplified calculators for estimating emissions are still adapted to national contexts. We performed an intra-national profiling of sheep dairy farms to verify whether distinct groups of emission intensity exist and which structural and technical variables are associated with the observed differences. Aggregate farm inputs were analysed for France (191), Italy (101) and Romania (92) using descriptive statistics, clustering on emission intensity expressed in kg CO<sub>2</sub>/kgFPCM, principal component analysis (PCA). The clustering consistently identified two completely separate groups: France 5.83 vs 3.34, Italy 5.53 vs 3.11 and Romania 8.67 vs 5.31 kg CO<sub>2</sub>e/kg FPCM. PCA revealed country-specific separation patterns. In France, PC1 and PC2 explained 55% of the variance (40% and 15.2%): PC1 represented farm size and production, while PC2 contrasted electricity per ewe with stocking density and organic nitrogen per hectare. In Italy, PC1 and PC2 explained 41% (27.2% and 13.9%): PC1 reflected herd size, standardised milk production and total energy, while PC2 was determined by allocation, fuel use and intensity per ewe. In Romania, PC1 and PC2 explained 44% (25.9% and 18.3%): PC1 was dominated by concentrate use and corrected milk production together with proxies for herd size, while PC2 was dominated by energy allocation and direct energy use. These preliminary results indicate that differences in emissions intensity arise from different combinations of scale/productivity, feed dependency and energy use, rather than from a single universal factor. For Mediterranean systems, linking cluster membership to its discriminating inputs (FPCM, concentrates and direct energy) translates segmentation into practical mitigation priorities within the system. This work was developed as part of LIFE Green Sheep (LIFE19 CCM/FR/001245).

### Greenhouse gas emissions from ruminant manure stockpiles in arid regions: mitigation strategies and microbial drivers

M. Omirou<sup>1</sup>, K. Philippou<sup>1</sup>, A. Phokas<sup>1</sup>, P. Pissaridou<sup>1</sup>, J. Rodosthenous<sup>1</sup>, C. Hadjimichael<sup>1</sup>, K. Kaikiti<sup>1</sup>, G. Hadjipavlou<sup>1</sup>, D. Sparaggis<sup>1</sup>

<sup>1</sup> Agricultural Research Institute, Agricultural Research Institute, 1516 Lefkosia, Cyprus

Ruminant manure stockpiling is a common practice in arid and semi-arid livestock systems, yet its contribution to greenhouse gas (GHG) and ammonia (NH<sub>3</sub>) emissions, and the microbial mechanisms regulating these fluxes, remain poorly understood under water-limited conditions. In a complete randomized design with 2 treatments (biochar and no-biochar) and 5 replicates we quantified methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), carbon dioxide (CO<sub>2</sub>), and NH<sub>3</sub> emissions from ruminant manure stockpiles to evaluate the biochar application as a mitigation tool. Gas fluxes were monitored at high frequency (daily measurements) during over a total storage duration of 180 days. Microbial processes were also assessed using quantitative PCR targeting functional genes involved in methanogenesis and nitrification–denitrification, complemented by shotgun metagenomics to characterise broader carbon and nitrogen cycling pathways. Treatment effects and temporal dynamics were analysed using linear mixed-effects models and repeated-measures ANOVA. Emissions were negligible during dry periods but increased sharply following re-wetting events, identifying moisture availability as a primary control of gaseous losses. Nitrification-related gene abundance increased during later storage stages, consistent with declining NH<sub>4</sub><sup>+</sup>, while denitrification-related gene abundance declined concurrently with increasing NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> availability. Biochar-amended stockpiles exhibited consistently lower N<sub>2</sub>O emissions throughout the storage period. This reduction was associated with significantly lower nirK abundance in biochar-treated manure, indicating reduced denitrification potential. NH<sub>3</sub> emissions peaked sharply in the untreated control during the first 10 days of storage, resulting in cumulative losses approximately four times higher than with biochar, whereas biochar induced a strong and sustained reduction in NH<sub>3</sub> emissions through ammonium adsorption and reduced volatilisation. In contrast, CH<sub>4</sub> emissions were largely unaffected by biochar, peaking early and declining to near-zero values by day 40 in both treatments.

## Session 7

## Theatre 6

### From management practices to technical performance in Italian dairy cattle farms

G. S. Sechi<sup>1,2,3</sup>, P. Bulgarelli<sup>4</sup>, F. Salati<sup>4</sup>, A. S. Atzori<sup>2,3</sup>

<sup>1</sup> University School for Advanced Studies IUSS Pavia, Piazza della Vittoria, 15, 27100 Pavia, Italy, <sup>2</sup> University of Sassari, Agricultural Sciences, Viale Italia 39, 07100 Sassari, Italy, <sup>3</sup> Gi-ANTLab Guided Innovation by Animal New Tech, SpinOff UNISS, P.Crispo 3, 07100 Sassari, Italy, <sup>4</sup> Gruppo Lactalis Italia, Via Flavio Gioia 8, 20149 Milano, Italy

Dairy farm sustainability requires integrating management practices across financial, organizational, and technical dimensions. While technical efficiency in dairy systems is extensively documented, the relationship between management practice adoption and farm performance remains underinvestigated. A survey was delivered in 148 Italian dairy cattle farms supplying Lactalis Italia, assessing 17 management variables across Financial Management, Training, Generational Transition, Organization, and Performance Monitoring. Practice adoption was measured on a 4-point scale; responses were merged with technical performance data from official audits including milk yield, animal welfare indices (from Classyfarm), and net carbon footprint. Statistical analysis employed Spearman correlations, Mann-Whitney U tests comparing adopters versus non-adopters, and chi-square tests for cessation intention. Correlation analysis revealed coherent clusters among related practices: monitoring practices (Income Over Feed Cost–pregnancy rate:  $\rho=0.55$ ), generational variables (under-40–succession planning:  $\rho=0.53$ ), and accounting tools ( $\rho=0.35$ – $0.40$ ). Farm digitalization showed strongest associations with productivity metrics (total milk yield:  $\rho=0.59$ ; milk per cow:  $\rho=0.52$ ). Mann-Whitney tests revealed significant performance differences: milk yield per cow was higher among adopters for paid leave (+2,152 kg), under-40 involvement (+1,558 kg), and pregnancy rate monitoring (+1,449 kg). Net carbon footprint was lower among adopters for paid leave:  $-0.20$  kg CO<sub>2</sub>-eq/kg FPCM. Milk quality showed no associations. Cessation intention was significantly higher among non-adopters for paid leave (28.3% vs 6.3%). Future research should employ longitudinal designs to establish causality and develop integrated decision-support tools combining financial and technical indicators.

### Maternal Heat Stress and Antioxidant Nutrition: Consequences for Early Gut Microbiome Maturation in Iberian Piglets

P. Vázquez-Ortego<sup>1</sup>, P. Aranguren-Rivas<sup>1</sup>, F. García<sup>1</sup>, A. García-Cabrero<sup>1</sup>, J. Segura<sup>2</sup>, Á. Olivares<sup>2</sup>, F. Sánchez-Esquiliche<sup>3</sup>, G. Gómez-Mateos<sup>4</sup>, C. Óvilo<sup>1</sup>, J. M. García-Casco<sup>1,5</sup>, A. Heras-Molina<sup>2</sup>, M. Muñoz<sup>1</sup>

<sup>1</sup> INIA-CSIC, Animal Breeding and Genetics Dept., 28040 Madrid, Spain, <sup>2</sup> UCM, Animal Production Dept., 28040 Madrid, Spain, <sup>3</sup> Sánchez Romero Carvajal, Ctra. San Juan del Puerto, s/n, 21290 Jabugo, Spain, <sup>4</sup> IRIAF, Ctra. Toledo-Albacete, s/n, 13700 Tomelloso, Spain, <sup>5</sup> INIA-CSIC, R+D Center in Iberian Pig, 06300 Zafra, Spain

Heat stress (HS) during gestation can compromise maternal microbial transmission and the establishment of the offspring's intestinal microbiota. The aim of this study was to investigate the impact of gestational HS on the gut microbiome of Iberian piglets whose mothers received different perinatal antioxidant supplementation as strategies to mitigate HS. Two trials were conducted: one during gestation from November to March (TN) and another from May to September (HS). A total of 72 Iberian sows per season were allocated to three dietary groups (n = 24) from day 50 of gestation until weaning: a) C (control), 100 IU vit E/kg and inorganic sources of minerals (Mn, Zn, Cu Se) according to FEDNA 2013; b) VE, 200 IU vit E/kg; and c) M, 45%–100% organic trace minerals. At 3 days of age, feces were collected from 157 HS piglets and 180 TN piglets. Fecal DNA was extracted and 16S rRNA gene was sequenced. Piglets gestated during HS showed lower  $\alpha$ -diversity than TN, and C diet also showed lower  $\alpha$ -diversity than antioxidant ones. Differential abundance analyses identified 61 different genera between HS and TN piglets, with more opportunistic taxa and fewer beneficial SCFA-producers under HS. VE showed higher abundance of bile- and sulfur-metabolizing taxa and propionate producers, and lower of opportunistic genera and butyrate producers than C. Overall, gestational HS seems to alter gut microbial diversity and composition that can be partially modulated by diet. Therefore, the results suggest that perinatal antioxidant nutritional strategies could be stabilizing early gut communities under HS conditions, which is particularly relevant for Mediterranean production systems where HS is recurrent. This work was financially supported by PID2022-139367OB-C21 and PREP2022-000441 funded by MICIU/AEI/10.13039/501100011033 (FEDER/UE, FSE+).

### Session 7

### Poster 9

#### Reuse of Treated Swine Wastewater in a Hydroponic Forage Production System

I. Rehan<sup>1,2,3</sup>, O. Moreira<sup>1</sup>, D. Murta<sup>4</sup>, F. Rebelo<sup>1</sup>, J. Costa<sup>1</sup>, K. Paulos<sup>1,2,3</sup>, F. Lidon<sup>2,3</sup>, A. Almeida<sup>5</sup>, M. D. F. Carvalho<sup>5</sup>, K. Silvério<sup>5</sup>, T. Santos<sup>5</sup>, I. Lopes<sup>6</sup>

<sup>1</sup> INIAV, Pólo de Inovação da Fonte Boa, Av. Prof. Vaz Portugal, Quinta da Fonte Boa, 2005-048 Vale de Santarém, Portugal, <sup>2</sup> NOVA School of Science & Technology, Campus of Caparica, 2829-516 Caparica, Portugal, <sup>3</sup> GeoBioTec Research Center, Campus of Caparica, 2829-516 Caparica, Portugal, <sup>4</sup> EntoGreen, Ingredient Odyssey SA, Industrial Zone of Santarém, 2005-079 Santarém, Portugal, <sup>5</sup> IPBeja, Escola Superior Agrária de Beja, 7800-309 Beja, Portugal, <sup>6</sup> Swedish University of Agricultural Sciences, Boz 190, 23422 Alnarp, Sweden

This study evaluated the feasibility of reusing treated swine wastewater (T-SWW) as an alternative irrigation and nutrient source for hydroponic green forage production. Wastewater underwent one-step lime precipitation followed by natural carbonation, producing a clarified, microbiologically safe effluent. Five forage species: oat, ryegrass, barley, durum wheat and triticale were cultivated for 9 days under 3 irrigation treatments: groundwater (control), a 1:1 mixture of groundwater and T-SWW and 100% T-SWW, in a controlled hydroponic system. Although T-SWW exceeded legal thresholds for some parameters (notably BOD<sub>5</sub> and total nitrogen), the treatment eliminated *Escherichia coli*, coliforms and intestinal parasite eggs. Plant growth, dry matter (DM) yield, water use efficiency (WUE) and nutritional composition were analysed using analysis of variance (ANOVA). No negative impacts were observed on plant growth or DM yield across treatments. Barley showed the highest WUE (0.95±0.03 kg/L), while wheat produced the greatest DM yield (4.7±0.1 kg/m<sup>2</sup>). Nutritional composition remained within acceptable ranges, with moderate variations in ash, crude protein and selected minerals (Na, K, Ca, Mn), depending on species. Results indicate that T-SWW can be integrated into hydroponic forage systems without compromising yield, nutritional quality or microbiological safety. This approach may support resilient livestock feeding by enabling on-farm forage production while valorising livestock effluents and reducing fresh-water demand. As a low-input system suitable for small-scale use, it represents a circular solution to improve resource efficiency and strengthen farm resilience in water-limited regions.

### Method Development for Monitoring Heat Stress in Small Ruminants Using Multiplex Digital PCR

J. Rodosthenous<sup>1</sup>, T. Christofi<sup>2</sup>, G. Hadjipavlou<sup>2</sup>, M. Omirou<sup>1</sup>

<sup>1</sup> Agricultural Research Institute, Agrobiotechnology, P.O.Box 22016, 1516 Nicosia, Cyprus, <sup>2</sup> Agricultural Research Institute, Animal Production, P.O.Box 22016, 1516 Nicosia, Cyprus

Heat stress is an escalating challenge for livestock production under global climate change, particularly in Mediterranean regions. Small ruminants, specifically the Cyprus Damascus goat and Cyprus Chios sheep, play a crucial role in dairy and meat production and exhibit relative heat resilience. However, increasing temperatures adversely affect their immunity, productivity, and reproduction. Robust molecular tools for assessing heat stress responses are therefore essential for developing climate-resilient breeding strategies. The primary objective is to develop a multiplex (9-plex) Crystal Digital PCR (cdPCR) assay using the Stilla Nio for absolute quantification of heat stress-related mRNA expression in sheep and goats. Primers and probes for eight stress-responsive genes and one housekeeping gene were designed in silico and optimized for 9-plex detection. The assay targets transcripts associated with acute heat stress (HSP70, HSP90, IL6) and chronic or adaptive responses (HSP27, SOD2, GPx, IL2, IL12B), using GAPDH as a reference to monitor RNA integrity and cDNA synthesis. A cohort of 82 animals: 42 female Cyprus Chios sheep and their offspring, was recruited at the ARI Athalassa Experimental Farm. Whole blood samples were collected in summer and winter to capture seasonal thermal variation. Peripheral blood mononuclear cells were isolated, followed by RNA extraction and cDNA synthesis. Each target was initially evaluated in a single-plex cdPCR to optimize cDNA input, after which the 9-plex cdPCR assay was tested. Preliminary results show high sensitivity and clear target discrimination, with optimization ongoing prior to large-scale application. This study aims to develop a precise and reproducible tool to assess acute and chronic heat stress responses and inter-individual thermotolerance in small ruminants. The assay facilitates correlation of seasonal molecular expression levels with phenotypic and production traits. Longitudinal multigenerational monitoring will evaluate the heritability of thermotolerance and support resilience-oriented breeding strategies to sustain productivity under increasing thermal stress.

## Session 7

## Poster 11

### The Andalusian turkey's rearing systems as an example of backyard poultry in Western Europe

J. I. Salgado Pardo<sup>1</sup>, A. González Ariza<sup>2</sup>, J. V. Delgado Bermejo<sup>1</sup>, I. Castro Castillo<sup>1</sup>, C. Barba Capote<sup>3</sup>, M. E. Camacho Vallejo<sup>4</sup>

<sup>1</sup> University of Córdoba, genetics Department, C5 Gregor Mendel building, Rabanales University Campus, 14071 Córdoba, Spain, <sup>2</sup> Diputación de Córdoba, Agropecuaria Provincial Centre, N-IV road, Km.396, 14071 Córdoba, Spain, <sup>3</sup> University of Córdoba, Animal Production Department, C5 Gregor Mendel building, Rabanales University Campus, 14071 Córdoba, Spain, <sup>4</sup> Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Department of Agriculture and Ecological Husbandry, IFPA Alameda del Obispo, 14004 Córdoba, Spain

Indigenous poultry genotypes have suffered genetic erosion and productive replacement by highly selected strains. However, they act as genetic diversity reservoirs and provide an easy household in rural and low-income communities. Furthermore, indigenous poultry genotypes represent local adaptation, since they are linked to the ancestral management and agroecological landscape of the local cultures that gave origin to them. The aim of the present study is to describe the traditional rearing systems in which the Andalusian turkey, a critically endangered local breed, has been maintained up to this day. To this goal, 10 of the few traceable Andalusian turkey breeders were surveyed following a 102 item-questionnaire addressing: breeder profile, facilities, farm access, feeding and reproductive management, and products' market. Most of the farmers surveyed were male, employed and had a high educational attainment. They bred the birds as a hobby without any economic expectation, but most sell birds for consumption and, in some cases, edible eggs. They live on the farms (40%) or closet to them (<6.75km). The farms commonly have no specialized facilities nor equipment for shelter or animal care. Most of the pastures are extensive olive groves (40%), courtyards (40%), and pastureland shared with other livestock species (20%). Animals base their intake on foraging, with minimal supplementation and low expenditure on medical, hygienic and nutritional support. The Andalusian turkey is raised alongside other endangered breeds of poultry and livestock from Andalusia. These farmhouses therefore act as isolated 'genetic biodiversity refuges', in harmony with the traditional Andalusian agricultural landscape.

### Lambing Performance of Sarda Ewes Inseminated with Liquid-Stored Semen at 4 °C for up to 72 Hours.

C. Nassif<sup>1,2</sup>, F. Chessa<sup>2</sup>, I. Cossu<sup>2</sup>, G. Epifani<sup>2</sup>, O. Todde<sup>1</sup>, L. Mara<sup>2</sup>

<sup>1</sup> Associazione Allevatori della Regione Sardegna (AARS), P.za Antonio Segni, 1, 07100 Sassari, Italy, <sup>2</sup> Agricultural Research Agency of Sardinia (Agris), Department of Animal Science, Localita' Bonassai Ss 291, KM. 18.600, 07100 Sassari, Italy

Mediterranean sheep systems face increasing climate and resource constraints, limited access to cryogenic infrastructure, and rising service costs for reproductive technologies. Ovine semen also has low tolerance to cryopreservation, and the ewe's complex cervix reduces the efficiency of cervical AI with frozen-thawed semen, often requiring laparoscopic intrauterine insemination. To support low-input, locally adapted and more resilient breeding programs, we evaluated liquid semen storage at 4 °C for up to 72 h in hormonally synchronized Sarda ewes inseminated at the first cervical fold. Semen from eight rams was collected, diluted with a commercial extender (OviXcell®), pooled, and stored at 4 °C. Eighty-one ewes were synchronized using a standard hormonal protocol and assigned to four storage-time groups: 7 h (G1; n = 21), 24 h (G2; n = 20), 48 h (G3; n = 20), and 72 h (G4; n = 20). Pregnancy rates were 66.7%, 75.0%, 45.0%, and 35.0%, while lambing rates were 57.1%, 60.0%, 30.0%, and 20.0% for G1–G4, respectively. Logistic regression (categorical time factor) showed significant group effects for pregnancy (Likelihood Ratio  $\chi^2 = 8.64$ , df = 3, p = 0.035) and lambing ( $\chi^2 = 10.14$ , df = 3, p = 0.017). Compared with 7 h, 72 h storage reduced the odds of pregnancy (OR = 0.27, p = 0.046) and lambing (OR = 0.19, p = 0.019). From a resilience and sustainability perspective, liquid semen avoids liquid-nitrogen supply and long-distance cold-chain dependencies and reduces reliance on laparoscopic AI. Estimated per-insemination costs may be €3–6 for liquid semen versus €10–15 for frozen semen programs (≈30–60% reduction). Overall, commercial extenders preserved fertility effectively up to 24 h; beyond 24 h, fertility declined but was not abolished. Further optimization of extender formulations could extend storage time and strengthen adaptive, low-infrastructure reproductive strategies for Mediterranean livestock farming systems.

### Session 7

### Poster 13

### Influence of maternal backfat thickness on Sarda piglet performance

C. Mussolino<sup>1</sup>, M. F. Lunesu<sup>1</sup>, M. Cocco<sup>1</sup>, A. Fenu<sup>1</sup>, G. Battacone<sup>1</sup>

<sup>1</sup> University of Sassari, Dipartimento di Agraria, Viale Italia 39a, 07100 Sassari, Italy

The Sarda pig is an autochthonous breed from Sardinia (Italy) characterized by remarkable rusticity and adaptation to extensive rearing systems. In this context, suckling pig production represents a distinctive feature of the system. Sow backfat thickness acts as an important energy reserve, which may influence lactation performance and, consequently, piglet growth. The aim of this study was to evaluate the effect of sow backfat thickness on the growth performance of Sarda suckling piglets. Thirty-five Sarda sows were classified into four groups based on third-level backfat thickness (BFt): Class 1 (12–21 mm, n=9), Class 2 (21–26 mm, n=9), Class 3 (26–34 mm, n=9), and Class 4 (34–40 mm, n=8). The backfat thickness was measured by ultrasonic probe before farrowing and during the first three weeks of lactation. Piglets body weight was recorded three times, from birth until weaning, to calculate the average daily weight gain (ADG). Litter size was also recorded. Sow daily milk yield was estimated throughout the lactation period. Data were analyzed using a linear mixed model (PROC MIXED, SAS) to evaluate the effects of BFt, recording time, and their interaction. No significant effects of BFt, recording time, or their interaction were observed on litter size, milk yield, birth weight, and ADG ( $P > 0.05$ ). On average, litter size was  $8.41 \pm 0.17$  piglets per sow (mean  $\pm$  SEM), and estimated milk yield was  $6.81 \pm 0.21$  kg/d. Mean piglet birth weight was  $1.16 \text{ kg} \pm 0.09$ , with an ADG of  $0.78 \pm 0.10$  kg/d during the first three weeks. These preliminary results evidenced that backfat thickness of Sarda sows did not significantly influence piglet growth performance. Probably, dietary intake was able to compensate for differences in body reserves among the sows. These partial results suggest the need to investigate the nutritional requirements, particularly protein needs, of Sarda sows during lactation. Indeed, body fat reserves do not appear to limit their milk production capacity. However, further research is needed to explore the interaction between body fat reserves and dietary protein on piglet growth and welfare.

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