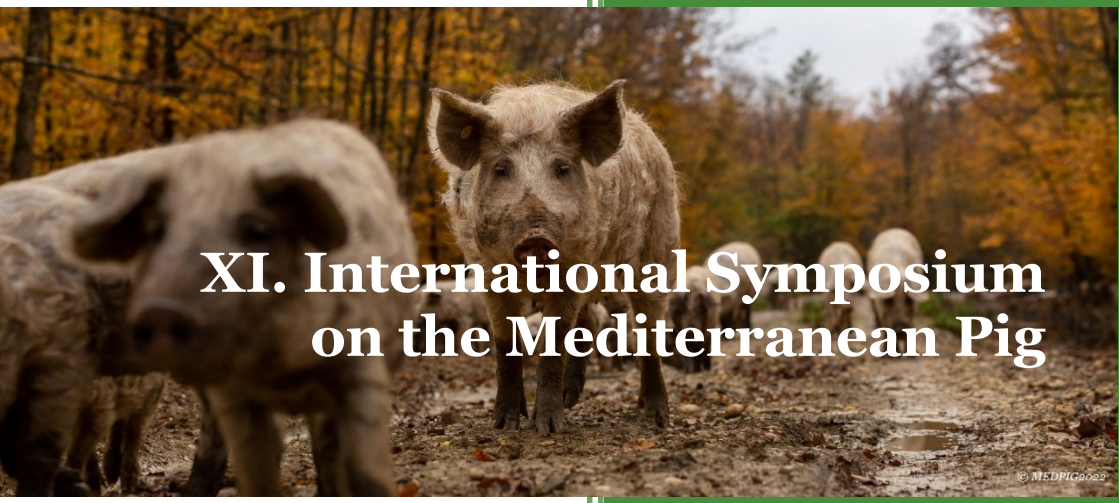
BOOK OF ABSTRACTS



XI. International Symposium on the Mediterranean Pig

October 11 – 14, 2022

Vodice, Croatia

XI International Symposium on the Mediterranean Pig

11-14 October 2022, Vodice, Croatia

BOOK OF ABSTRACTS

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INDEX

FINAL PROGRAMME

Venue	Hotel *Olympia SKY* ****+	
Tuesday, 11.10.2022		
9:00 - 12:00 15:00 - 20:00	**ARRIVAL - REGISTRATIONS**	
Wednesday, 12.10.2022		
9:00 - 9:30	**OPENING CEREMONY** *Welcome addresses*	
9:30 - 11:00	**Session 1. Animal Breeding and Genetics (Part 1)**	
Chairs	Riccardo Bozzi & María Muñoz	
9:30 - 10:00	**MAIN LECTURE** Cristina Óvilo **Genetic and genomic association studies in Iberian pigs**	M1.1
10:00 - 10:15	Valeria Taurisano, Anisa Ribani, Matteo Bolner, Mohamad Ballan, Samuele Bovo, Giuseppina Schiavo, Luca Fontanesi **Variability of nuclear mitochondrial DNA regions in the genome of autochthonous and cosmopolitan pig breeds**	O1.1

10.15 - 10.30	María Muñoz, Juan María García-Casco, Patricia Palma-Granados, Fabián García, José Manuel Martínez Torres, María Elena González-Sánchez, Juan Florencio Tejeda **Loin transcriptome analyses in Iberian pigs divergent for meat quality traits**	O1.2
10:30 - 10:45	Giuseppina Schiavo, Samuele Bovo, Anisa Ribani, Valeria Taurisano, Maria Muñoz, Cristina Óvilo, TREASURE Consortium, Maurizio Gallo, Luca Fontanesi **Boruta algorithm implementation for the classification and allocation of European pig breeds based on high density single nucleotide polymorphisms**	O1.3
10:45 - 11:00	Samuele Bovo, Giuseppina Schiavo, Valeria Taurisano, Anisa Ribani, Mohamad Ballan, Matteo Bolner, Luca Fontanesi **Mining whole genome sequencing data from more than 600 pigs of different breeds to identify variants in coat colour genes**	O1.4
11:00 - 11:30	**Coffee-break**	

11.30 - 13.30	**Session 2. Production Systems, Feeding and Environment**	
Chairs	Rosa Nieto & Zoran Luković	
11.30 - 12.00	**MAIN LECTURE** Nina Batorek Lukač, Marjeta Čandek Potokar **Growth potential of Krškopolje pigs in different production systems**	M2.1
12:00 - 12:30	**MAIN LECTURE** Krešimir Salajpal, Oliver Martinić, Sven Menčik, Dubravko Škorput, Zoran Luković, Danijel Karolyi **Environmental impact of traditional low-input pig production systems**	M2.2
12:30 - 12.45	Vladimir Živković, Marija Gogić, Nenad Stojiljković, Čedomir Radović, Radomir Savić, Dragan Radojković, Aleksandra Petrović **Production systems and feeding of local breeds of pigs in Republic of Serbia**	O2.1
12.45 - 13.00	Zoran Luković, Dubravko Škorput, Krešimir Salajpal, Sven Menčik, Danijel Karolyi **Banija spotted pig – from science to practise**	O2.2

13:00 - 13.15	Mercedes Izquierdo, Nicolás Garrido, Antonio Gonzalez-Bulnes, Javier García-Gudiño, Miguel Angel Pérez, Francisco I. Hernández **Interactions between three Iberian pig strains and two feeding systems for changes in blood metabolic parameters during finishing**	O2.3
13.15 - 13.30	Rosa García-Valverde, Manuel Lachica, Patricia Palma-Granados, Ana Haro, Luis Lara, Ignacio Fernández-Fígares, Rosa Nieto **Effects of dietary tannins on parotid gland growth and saliva composition of Iberian pigs**	O2.4
13:30 - 15.00	**Lunch-break**	
15.00 - 16.15	**Session 3. Animal Health, Physiology, Welfare and Reproduction**	
Chairs	Ratky József & Nina Batorek Lukač	
15.00 - 15.30	**MAIN LECTURE** Somoskői Bence, Egerszegi István, Török Dóra, Albert Fruzsina, Nagy Szabolcs-Tamás, Bodó Szilárd, Benedek Zsuzsa, Tóth Péter, Rátky József **In vitro gene preservation of Hungarian Mangalica pig in the shadow of ASF**	M3.1

15.30 - 15.45	Soraya Solar-Malaga, Luis J García-Marín, Mercedes Torres-Badia, David Martin-Hidalgo, M. Julia Bragado **Antibiotic-free hypothermic storage of Iberian boar semen: in vitro sperm function and bacterial load**	O3.1
15.45 - 16.00	María Muñoz, Adrián López-García, Patricia Palma-Granados, Ana María García-Cabrero, Fabián García, Gerardo Gómez, Gema Matos, Cristina Óvilo, Juan María García-Casco **Analyses of heat stress effects on the vagina microbiome of Iberian sows**	O3.2
16.00 - 16.15	Sven Menčik, Ivan-Conrado Šoštarić-Zuckermann, Branka Artuković, Ivana Mihoković Buhin, Lea Grbavac, Mario Ostović, Anamaria Ekert Kabalin **Leg weakness, osteomalacia and hypocalcemia in weaned Black Slavonian piglet: A case report**	O3.3
16.15 - 16.30	**Coffee-break**	
16:30 - 18.00	**Poster Session**	
Chairs	Dubravko Škorput & Klavdija Poklukar	
20.00	**Welcome Cocktail** Presentation of local pork products	

Thursday, 13.10.2022		
9:00 - 11:00	**Session 4. Meat and Product Quality, Innovations and Traceability**	
Chairs	Ivona Djurkin-Kušec & José Manuel Martins	
9:00 - 9.30	**MAIN LECTURE** Martin Škrlep, Klavdija Poklukar, Bertrand Servin **Underlying mechanisms of lipid deposition in local and modern pig breeds**	M4.1
9:30 - 10:00	**MAIN LECTURE** Goran Kušec, Ivona Djurkin Kušec, Kristina Gvozdanović, Danijela Samac **Different understandings of pork quality - a historical overview**	M4.2
10:00 - 10:15	José Manuel Martins, André Albuquerque, Maria João Rodrigues, José Neves, Amadeu de Freitas, José Tirapicos Nunes, Rui Charneca **Loin and fat quality of Portuguese local Alentejano and Bísaro pigs and their crosses**	O4.1
10.15 - 10.30	Klavdija Poklukar, Marjeta Čandek Potokar, Nina Batorek Lukač, Urška Tomažin, Martin Škrlep **Biochemical and gene expression differences associated with higher fat deposition in Krškopolje pigs – comparison with lean crossbred pigs**	O4.2

10.30 - 10.45	Suzana Krhlanko, Anita Ule, Andrej Kastelic, Milena Kovač, Špela Malovrh **Implementation of traceability in Krškopolje pig**	O4.3
10:45 - 11:00	Sandra Petričević, Irena Listeš, Eddy Listeš, Damir Lukačević, Zdravka Vidić, Tomislav Dujić, Ines Skoko, Anđelo Katić, Ante Madir, Tanja Bogdanović **Sensory profile of Croatian dry-cured hams: PDO (Istarski Pršut) and PGI (Krčki, Dalmatinski and Drniški Pršut)**	O4.4
11:00 - 11:30	**Coffee-break**	
11:30 - 13:15	**Session 1. Animal Breeding and Genetics (Part 2)**	
Chairs	Cristina Óvilo & Francesco Tiezzi	
11:30 - 12:00	**MAIN LECTURE** Riccardo Bozzi, Francesco Tiezzi **Conservation of Italian local pig breeds: trick or treat?**	M1.2
12:00 - 12:15	Dubravko Škorput, Danijel Karolyi, Krešimir Salajpal, Marija Špehar, Zoran Luković **Optimal contribution selection – tool for sustainable breeding in small pig populations**	O1.5

12:15 - 12:30	Anita Ule, Suzana Krhlanko, Andrej Kastelic, Milena Kovač, Špela Malovrh **Pedigree verification and parentage assignment using genomic information in Krškopolje pig**	O1.6
12:30 - 12:45	Maria Chiara Fabbri, Emmanuel Lozada-Soto, Francesco Tiezzi, Alessandro Crovetti, Silvia Parrini, Francesco Sirtori, TREASURE Consortium, Riccardo Bozzi **Can we get rid of autozygosity in the crossbred pig?**	O1.7
12:45 - 13.00	Juan María García-Casco, Patricia Palma-Granados, Miguel Ángel Fernández-Barroso, Carmen Caraballo, Fernando Gómez-Carballar, Fernando Sanchez Esquiliche, Cristina Óvilo, Yolanda Núñez, María Muñoz **Association results in different Iberian pig populations between SNPs and phenotypes related to meat quality traits**	O1.8
13:00 - 13:15	Anisa Ribani, Valeria Taurisano, Samuele Bovo, Giuseppina Schiavo, Stefania Dall'Olio, Silvia Tinarelli, Maurizio Gallo, Luca Fontanesi **Monitoring major gene polymorphisms in Italian pig breeds**	O1.9
13:15 - 15.00	**Lunch-break**	
15.00 - 16.15	**Session 5. Socio-Economic Aspects**	
Chairs	Marjeta Čandek Potokar & Marie-José Mercat	

15:00 - 15:30	**MAIN LECTURE** Marie-José Mercat, Andreia Amaral, Riccardo Bozzi, Marjeta Čandek-Potokar, Pedro Fernandes, Jetsabell Gutierrez Vallejos, Danijel Karolyi, Denis Laloë, Zoran Luković, Gwendal Restoux, Virginia Ribeiro, Tamara Rodríguez, Romuald Rouger, Dubravko Škorput, Martin Škrlep, António Vicente **Stakeholders' perception of the local breeds' sector in six European countries - a survey by the GEroNIMO project**	M5.1
15:30 - 15:45	Giuseppe Bonazzi, Mattia Iotti, Federica Bonazzi **Protection of biodiversity and breeding of the Black Pig of Parma and of the Cinta Senese PDO in the Tuscan-Emilian Apennines**	O5.1
15:45 - 16:00	Sanja Jelić Milković, Ružica Lončarić, Igor Kralik, Goran Kušec, Ivona Djurkin Kušec, Maurizio Canavari **Consumer's opinions about societal concerns when purchasing fresh pork from the Black Slavonian Pig**	O5.2
16:00 - 16.15	Danijel Karolyi, Juraj Odrčić **Protected Designation of Origin (PDO) label for pork of local breeds - the case of Turopolje pig**	O5.3
20:00	**Gala dinner**	

Friday, 14.10.2022		
9:00 - 16:00	**Field trip** Visit to a local ham factory BEL-CRO, Brištane Visit to the National Park Krka Lunch at the Atrium Gulin restaurant, Lozovac	
Session	**LIST OF POSTERS**	
Animal Breeding and Genetics	Yolanda Núñez, Gerardo Gómez, Hernán Dario Laviano, Fabián García, María Muñoz, Juan María García-Casco, Rita Benítez, Fernando Sánchez-Esquiliche, Antonio González-Bulnes, Ana Isabel Rey, Clemente Jose López-Bote, Cristina Óvilo **Antioxidants addition in the diet of pregnant sows modifies the expression of genes related to ovarian functionality in their progeny**	P01
Production Systems, Feeding and Environment	José Manuel Martins, Rui Charneca, Ricardo Varino, André Albuquerque, Amadeu de Freitas, José Neves, Filipa Costa, Carla Marmelo, Amélia Ramos, Luísa Martin **Outdoor finishing of intact male pigs of local breed on a high fibre diet: effects on growth, carcass, and some meat quality traits**	P02

Production Systems, Feeding and Environment	Javier García-Gudiño, Eudald Llauradó-Calero, Mercedes Izquierdo, Nuria Tous, Francisco I. Hernández-García **Effect of fish oil supplementation of the Iberian sow on piglet performance during lactation**	P03
Production Systems, Feeding and Environment	Vittoria Asti, Alberto Sabbioni, Francesco Delmonte, Elena Mariani, Michela Ablondi **Animal and environmental factors affecting reproductive traits in Nero di Parma sows**	P04
Production Systems, Feeding and Environment	Zaira Pardo, Rosa Nieto, Gloria González-Cañas, Manuel Lachica, Luis Lara, Isabel Seiquer, Ignacio Fernández-Fígares **Effect of long term heat stress on nutrient digestibility of Iberian pigs**	P05
Production Systems, Feeding and Environment	Carolina Pugliese, Chiara Aquilani, Santo Carpino, Lapo Nannucci, Andrea Confessore, Riccardo Bozzi **The Apulo-Calabrese pig breed: a valuable scientific, economic, and social heritage**	P06
Production Systems, Feeding and Environment	Vladimir Margeta, Kristina Gvozdanović, Ivona Djurkin Kušec, Danijela Samac, Goran Kušec, Žarko Radišić, Dalida Galović **"Green deal" as a framework for the development of sustainable pig production in Croatia**	P07

Animal Health, Physiology, Welfare and Reproduction	Nina Batorek Lukač, Marjeta Čandek Potokar, Gregor Fazarinc, Martin Škrlep, Klavdija Poklukar, Milka Vrecl **Physiological effects of androgen deprivation in the late sexual development phase (model of adult boars)**	P08
Animal Health, Physiology, Welfare and Reproduction	Francisco I. Hernández-García, Javier García-Gudiño, M. Victoria Alarcón, Alicia Flores-Roco, Ana Hurtado, María Alejo, Nicolás Garrido, Dolores Ayuso, Manuel Morano, Ana I. del Rosario, Miguel A. Pérez, Mercedes Izquierdo **Effect of arginine supplementation in the Iberian pig on performance during the lactation and post-weaning periods**	P09
Meat and Product Quality, Innovations and Traceability	Chiara Aquilani, Francesco Sirtori, Lapo Nannucci, Andrea Confessore, Francesco Tiezzi, Carolina Pugliese **Effect of genetic type on tissue composition and meat quality traits in Nero di Parma pig breed and its cross with Casertana**	P10
Meat and Product Quality, Innovations and Traceability	Radomir Savić, Dragan Radojković, Marija Gogić, Nenad Stojiljković, Vladimir Živković, Aleksandra Petrović, Čedomir Radović **Carcass traits of Mangalitsa pigs – effect of immunocastration**	P11

Meat and Product Quality, Innovations and Traceability	Marko Bagarić, Kristina Gvozdanović, Ivona Djurkin Kušec, Miodrag Komlenić, Velimir Sili, Žarko Radišić, Goran Kušec **Influence of on-farm resting time on some meat quality parameters in sows**	P12
Meat and Product Quality, Innovations and Traceability	Diego Tejada, Juan Florencio Tejeda, Juan M. García, Elena González **Intramuscular fat contents in Iberian pig: Relationship with productive performance and *in vivo* estimation by ultrasound technique**	P13
Meat and Product Quality, Innovations and Traceability	Francesco Tiezzi, Maria Chiara Fabbri, Alessandro Crovetti, Silvia Parrini, Lapo Nannucci, Riccardo Bozzi **A meta-analysis of fat composition in Cinta Senese and its crosses**	P14
Meat and Product Quality, Innovations and Traceability	Danijel Karolyi, Sandra Petričević, Zoran Luković, Dubravko Škorput, Krešimir Salajpal, Tanja Bogdanović, Marjeta Čandek-Potokar **Influence of smoking method and anatomical site on the content of polycyclic aromatic hydrocarbons in Turopolje ham**	P15
Meat and Product Quality, Innovations and Traceability	Francesco Sirtori, Chiara Aquilani, Mariachiara Fabbri, Andrea Confessore, Silvia Parrini, Alessandro Crovetti, Carolina Pugliese **Alternative packaging techniques for the valorization of Cinta Senese dry-cured ham**	P16

	PROJECT PRESENTATIONS	
Horizon 2020 GA No 952303	**AgriFoodBoost – economic experiments applied to agriculture, food and environment**	P17
Horizon 2020 GA No 101000236	**GEroNIMO - Genome and Epigenome eNabled breedIng in MOnogastrics**	P18

M - Main lecture; O -Oral presentation; P – Poster/project presentation

SESSION 1

ANIMAL BREEDING AND GENETICS

MAIN LECTURES

Genetic and genomic association studies in Iberian pigs

Cristina Óvilo

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Classical genetic selection methods have limitations, especially in addressing the improvement of traits such as those related to meat quality parameters due to different factors, including their costly and late registration or the antagonisms of these characters with those of efficiency and performance. In this context, advances in molecular genetic analysis technologies can allow the design of more precise and effective breeding programs, based on the use of genetic markers. Besides, molecular genetic studies can help in deepening on the knowledge of the biological basis of relevant traits and physiological processes. Molecular studies on candidate genes have allowed the identification of some mutations with relevant effects, although just in a few cases they are considered causal of the effects found. Among those, LEPR, SCD or PCK1 gene markers can be highlighted for their association with fat deposition and meat quality traits. Moreover, in the last years the development and accessibility of high throughput sequencing and genotyping technologies have contributed to the comprehensive and genome-wide search of relevant markers and genomic regions affecting relevant productive traits in all livestock species. These tools have been also employed in Iberian pigs. We have employed a combined approach of candidate SNP recovery from literature and SNP discovery from whole genome sequencing and RNAseq data which has allowed the validation of the phenotypic effects of known candidate genes, as well as the identification of many other relevant genes not yet known to be involved in productive traits. Moreover, this multiomics data mining has allowed to highlight the usefulness of transcriptome deep-sequencing data in the detection of useful and relevant SNPs for the discovery of untapped genetic basis of productive traits. Also, a genome-wide association study (GWAS) performed in pure Iberian pigs has led to the identification of genomic regions associated to important traits involved in ham quality and yield,

backfat thickness, fatty acid composition and activity of several desaturase enzymes in different muscles. New positional and biological candidate genes can be proposed, located within the GWAS significant regions. The results of these works provide a valuable basis for a better understanding of the molecular genetic basis of relevant traits in pigs and provide new interesting SNPs and candidate genes for association studies in different pig breeds and populations and for future implementation of marker-assisted selection strategies.

Keywords: Iberian pig, genomics, SNP, candidate gene, association study, fatness, growth, meat quality

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Conservation of Italian local pig breeds: trick or treat?

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Attention to the conservation of animal genetic resources can be considered acquired by scientific community and institutions. Information on swine is now available on the main parameters of genetic diversity for many breeds with both limited and non-limited population size. Common belief is that local breeds possess characteristics of resistance and adaptation that are not found in cosmopolitan breeds. However, these traits are difficult to measure and, to our knowledge, are not under selection or even under phenotypic control. In Italy, for instance, each local pig breed has its own Herd Book with a specific conservation program. The actions that are undertaken concern the control of inbreeding and the conservation of sufficient genetic variability combined with a phenotypic and, in some cases, genetic characterization activity to retain some peculiar characteristics. Other than these 'breed standards' to adhere to, breeding goals are absent. However, the pursuit of keeping the breed standards (such as the characteristic 'white belt' in the Cinta Senese breed, for example) leads to the application of non-null selection intensity: in fact, several piglets per litter often do not comply with the defined standards and such standards are based on inherited characteristics. This management and the diffusion at regional level of the breeds, have meant that the numerical consistency of the same never increased to such numbers in order to consider them no longer at risk of genetic erosion. Both, limited size and spread of populations also makes them particularly vulnerable in case of health emergencies such as the African Swine Fever currently present in Italy. At the same time, the use of the local breeds for crossbreeding with the cosmopolitan ones is gaining relevance, due to the interest of the farmers in exploiting their combining ability and hybrid vigour. The farmers' interest suggests that other uses

for these breeds are possible, other than pure-breeding. This could help fitting this germplasm on the geographical and economical area of origin. The conservation of these local breeds certainly passes through the commercial enhancement of the products, producing in some cases a remarkable development of the populations. It would also be necessary to set up both selection programs aimed at the search for those characteristics of resilience mentioned above and towards the use of breeding animals for the creation of crossbred-derived products that should combine the positive characteristics of the local breeds with those of cosmopolitan ones. To date, the use of optimal contribution selection (OCS) makes possible to implement selection programs with optimal constraints in terms of minimum genetic variability allowed for a given genetic progress, providing that resilience traits will be available. On the other hand, the correct management of the crossed products requires to implement a genotyping plan of the breeding individuals to identify the favourable combinations in terms of both genetic variability and heterozygosity. The same local breed could show different levels of hybrid vigour when crossed to different cosmopolitan breeds, due to genetic distance between the breeds and the different haplotype frequency within the breeds. The persistence of autozygosity in the crossbred individual could be controlled using information on the genetic distance between the breeds, the genetic variability within the breeds and the selection of the individuals to mate from each breed. Thus, which breeds and which individuals (within breed) to cross is not a trivial choice and should be carefully planned to exploit hybrid vigour at its best. In conclusion, the management of local pig European breeds is a complex task. While the conservation of biodiversity and their use in niche markets are paramount, the local breeds could find further use as a gene pool carried in a population well adapted to outdoor farming systems in marginal areas.

Keywords: Italian local pigs, optimal contribution selection, crossbreeding, homozygosity

SESSION 1

ANIMAL BREEDING AND GENETICS

ORAL COMMUNICATIONS

Variability of nuclear mitochondrial DNA regions in the genome of autochthonous and cosmopolitan pig breeds

Valeria Taurisano[1], Anisa Ribani[1], Matteo Bolner[1], Mohamad Ballan[1], Samuele Bovo[1], Giuseppina Schiavo[1], Luca Fontanesi[1]

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Insertions of mitochondrial DNA (mtDNA) into the nuclear genome have been detected in many eukaryotes. These sequences, originated by horizontal transfer of mtDNA fragments of both coding and non-coding regions, produces nuclear DNA sequences of mitochondrial origin (NUMTs). Similarly, to other inserted elements, NUMTs have been used to reconstruct the history of animal domestication and breed development at the genome level. For the same objective, in this study, we explored the potential informativity of NUMT regions' variability in six autochthonous Italian pig breeds (Apulo Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano and Sarda) and three Italian heavy pig breeds (Italian Landrace, Italian Large White and Italian Duroc). We initially detected NUMTs by mining the *Sus scrofa* reference genome (Sscrofa11.1) and several additional drafted pig genomes, including those assembled for several breeds. BLAST tool was used to map mtDNA sequences over the nuclear DNA genome. We then integrated this information by mining whole genome sequencing (WGS) data from DNA pools including several pigs or from single pigs. On the whole, about 700 NUMTs were retrieved by mining the assembled reference genome versions, 74% of which were also detected by mining WGS datasets. A total of 36 novel insertion events (*de novo* NUMTs, which were not reported in any assembled reference pig genome) were identified by evaluating discordant mapped reads and/or soft clipped reads. A total of 21 primer pairs, targeting six NUMTs detected from the reference genome and 15 *de novo* NUMTs, were designed to validate these regions by PCR and Sanger sequencing. PCR amplification was obtained in a

panel of 30-35 pigs of several breeds, for a total of >300 analysed animals. As expected from the *in silico* analyses, we confirmed the presence at high frequency of several targeted NUMTs in all analysed breeds. A few NUMT insertions were highly frequent (or almost private) in some breeds and can be very informative in the reconstruction of the domestication process of *Sus scrofa* and in the definition of the genetic history of many pig breeds. Overall, this study provided an updated porcine genome map of NUMTs. We also demonstrated that several NUMTs are polymorphic in some breeds (i.e. presence/absence of the insertion) and that, for this reason, can constitute interesting markers to trace back the origin of pig populations.

Keywords: Domestication, genome evolution, mitochondrial DNA, *Sus scrofa*

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Loin transcriptome analyses in Iberian pigs divergent for meat quality traits

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In the traditional Iberian pig production, some animals are fattened in extensive or open-air intensive system, where they use a large territory and they can even have access to seasonal resources such as grass, straw or grains although their main food source usually are feeds. This feeding system has been incorporated in the Spanish law (*Norma de Calidad*) with the label of "*cebo de campo*". The INIA-CSIC research group in collaboration with AECERIBER, COVAP and *Fundación La Contienda* (*La Caixa*) is developing a breeding program with "*cebo de campo*" pigs in which the study of the potential use of molecular markers to improve meat quality traits is included. One approach to study candidate genes for meat quality traits consists in sequencing the transcriptome of individuals divergent for a particular trait or a set of traits and carrying out differential expression analyses. The objective of this study was first, to detect pigs divergent for a set of meat quality traits and, second, sequence the transcriptome (RNAseq) of the loin muscle of these animals and perform differential expression analyses. Tenderness, water losses, color and intramuscular fat content was measured in 258 "*cebo de campo*" pigs fattened in three batches, the animals of each batch were born from the same group of sows and were slaughter altogether. Principal component analyses were carried out to group the pigs divergent for

meat quality traits. Six pigs from each divergent group, High (H) and Low (L) meat quality groups were selected, being the means of intramuscular fat, color a* (redness), shear force and thawing water losses equal to 8.65, 11.26, 2.39 and 2.97, respectively in H and 3.00, 9.13, 4.87 and 5.40 in L*. To avoid batch effects, two individuals per batch were included in each divergent group. Loin RNA was extracted and the samples were sequenced in Illumina NovaSeq 6000 equipment. Read quality was checked and trimmed according to standard criteria and the posterior bioinformatics analyses were carried out with the Hisat2-HTseq-counts-DESeq2 pipeline, mapping the reads against Sscrofa11.1 assembly. *In silico* functional analyses were carried out with EnrichR and Cluster profile tools. An average of 93.05% of the reads was concordantly aligned with Sscrofa11.1 assembly. A total of 13,087 out of 14,146 transcripts corresponded to protein coding genes. Fattened batch was included as fixed effect in the differential expression analysis, as a result, 223 genes were differentially expressed (DEGs), being 172 overexpressed in the H group and 51 in the L group. Among them, several genes involved in lipid metabolism, namely *APOE*, *ELOVL5*, *ELOVL6* and *SCD*, were DEGs also observed in other meat quality trait studies. The functional enrichment analysis revealed, among others, the following biological processes: extracellular matrix organization (GO:0030198), tissue morphogenesis (GO:0048729) and extracellular structure organization (GO:0043062) related with tissue development, and regulation of lipid metabolic process (GO:0019216). The results of the present study provide a set of candidate genes for meat quality traits and important information about the genetic mechanisms underlying meat quality traits in "*cebo de campo*" Iberian pigs.

Keywords: Meat quality, Iberian pigs, RNAseq, lipid metabolism

Acknowledgement

The research was funded with the grant IB18069, from the *Junta de Extremadura* and "*FEDER Una manera de hacer Europa*", and the agreement INIA-AECERIBER-COVAP-LA CONTIENDA CC19-156.

Boruta algorithm implementation for the classification and allocation of European pig breeds based on high density single nucleotide polymorphisms

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Autochthonous pig breeds are important reservoirs of genetic variability that can be exploited to identify alleles that may confer adaptation to peculiar production environments and climatic conditions. Genetic integrity of these breeds is a matter of concern of many conservation programs in European countries. High-throughput genotyping of single nucleotide polymorphisms (SNPs) is an efficient approach to capture the differences among breeds and then evaluate optimal management strategies for the conservation of this diversity. Several classification methods based on feature selection are available for SNP data but their implementation is often limited by computational resources. To overcome the computational bottleneck, the selection of a small number of genetic features has been proposed even if this approach can lose some biologically relevant elements. The aim of this work was to explore different marker panels to identify the proper balance between these aspects (i.e. computational burden and selection of informative SNPs) and to find good classification methods to differentiate several European autochthonous pig breeds. As Random Forests (RF) provided interesting results in previous works, Boruta algorithm (a RF wrapper method) was tested to select the more stable features (i.e. SNPs) after a series of

iterations. In this work, Boruta algorithm was applied on a total of 1154 pigs from 23 European breeds (48 per breed), genotyped with the GGP 70k Porcine array. Quality check and filtering were performed with PLINK1.9, which retained 16107 SNPs for feature selection. Analyses were carried out with *randomForest* and *boruta* R packages. Different numbers of iterations, from a minimum of 1000 to a maximum of 100000, were tested and markers labelled as "Confirmed" with Boruta analyses were chosen. Several subsets of SNPs have been obtained by recursively splitting the panel of Confirmed SNPs. Goodness of prediction was evaluated with the Out of Bag (OOB) error. A total of 2471 features was initially labelled as stable. The higher number of Confirmed SNPs (193) was on porcine chromosome (SSC) 8, while the minimum (41) was on SSC1. The smaller subset to obtain an OOB error < 1% included 171 SNPs. Annotation of genomic regions encompassing selected SNPs was carried out with Ensembl Biomart. According to the gene function in these regions, the selected markers may reflect the differences among breeds originated by natural or artificial selection.

Keywords: Autochthonous breed, breed classification, feature selection, random forests, SNP

Acknowledgements

This work has received funding from the University of Bologna RFO programs and from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 634476 for the project with acronym TREASURE.

Mining whole genome sequencing data from more than 600 pigs of different breeds to identify variants in coat colour genes

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Coat colour has been one of the first traits that was selected over the domestication process in all livestock species. This selection acted indirectly at the genome level where variants have been fixed or almost fixed in many breeds. The domestication process of the pig started from Asian, Middle East and European wild boar ancestors and spread mainly in China and Europe. Therefore, subsequently coat colour affecting genes have followed two main independent selection processes creating a quite high level of variability. Many genes are known to control pigmentation in mammals, including genes (i) affecting melanocytes morphology, development and migration, (ii) coding for melanogenic enzymes and (iii) affecting the melanosomal structure and function. In this study, we selected 25 coat colour genes, involved in all relevant biological processes related to pigmentation, and investigated their variability by mining more than 600 whole genome sequencing (WGS) datasets produced from 35 *Sus scrofa* breeds/populations and related species (*S. cebifrons*, *S. celebensis* and *S. verrucosus*). About 500 WGS datasets from cosmopolitan and European and Asian autochthonous pig breeds were retrieved from the European Nucleotide Archive. Additional 100 WGS data were produced from three Italian heavy pig breeds. Reads were mapped to the reference genome with BWA and polymorphisms were detected with Bcftools in the selected genes, considering a region spanning 5 kb upstream and 5 kb downstream the corresponding gene coordinates. Variants were annotated using the Variant Effect Predictor tool and SIFT was used for predicting the impact on the protein function.

In total, we analysed more than 10 Terabyte of sequencing data. A total of more than 150 k variants were identified, including about 7 k insertion/deletions (~5%). About 90% of all these variants were located outside the protein coding region (~80% in introns, ~10% in UTRs and ~10% flanking UTRs). Few variants might putatively affect the protein structure: about 600 and 200 missense variants were predicted by SIFT as tolerated and deleterious, respectively; about 30 variants determined stop codons or frameshifts. Allele frequencies for all variants were estimated across the different breeds. Variants were compared to establish their relationships with the known phenotypes and to confirm their putative role as causative variants. Variants were used to infer the coat colour of the pigs from which WGS data were produced and allocate animals to their breeds. Moreover, many variants were also used to verify the breed of origin of pork products.

Keywords: Genomics, pigmentation, *Sus scrofa*

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Optimal contribution selection – tool for sustainable breeding in small pig populations

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Local breeds of pigs are often characterised by low genetic diversity due to small population sizes and bottlenecks in populations. However, conservation processes for local breeds aim to maintain genetic diversity of the population and breeding programmes rarely include genetic evaluation for economically important traits. Conservation processes in local pig breeds usually rely on public financial support. However, to achieve sustainable management of breeds, breeding associations should establish breeding objectives that allow for self-sustainable breeding. From this point of view, genetic evaluation is necessary to achieve the breeding objectives and define specific characteristics of the breed. Maintaining genetic diversity and genetic progress in economically important traits are conflicting breeding objectives, especially when genetic evaluation is based on an animal model that tends to increase inbreeding by selecting more related animals. Optimal contribution selection (OCS) is an adequate approach that may obtain a balance between conflicting breeding objectives. However, the practical application of OCS in pig populations is not widespread. Few studies have been published on this topic based on simulations. Pedigree and molecular information can be used to obtain information on breeding values and average relatedness in the population. Several practical problems arise in the selection of local pig breeds, such as incomplete pedigree and data information, low connectedness between herds, low motivation of breeders to participate in the breeding programme, or high genotyping costs. OCS uses algorithms to find the best solutions between genetic gain and increase of inbreeding in the population. Different types of

algorithms have been introduced and applied for the optimization process, such as evolutionary and deterministic algorithms or solvers for linear, quadratic, and rational programmes. The preliminary results of OCS implementation on litter data for local Black Slavonian pig showed that it is possible to balance the genetic gain in the number of piglets born alive and the loss of genetic diversity, despite challenges derived by the poor quality of pedigree data. To this end, it is important to assign selection candidate status only to animals with complete phenotype information and sufficient pedigree quality. This results in a smaller number of selection candidates but ensures the reliability of the estimated relationships in the population and thus a balance between genetic gain and the loss of genetic variability.

Keywords: Pigs, genetic diversity, selection, optimal contributions

Pedigree verification and parentage assignment using genomic information in Krškopolje pig

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The purpose of this paper is to present the procedure for verifying parentage with genomic data in Krškopolje pigs (KP). The genomic data has facilitated verification of candidate parents, thus enabling more accurate pedigrees for mating plans, genetic evaluation, and a tool for resolving conflicts in the trading of breeding animals. To design an efficient preservation program for KP breed based on genetic evaluation for animals, accurate estimates of the population's genetic parameters are required. All pedigree data should be correctly recorded to avoid biased evaluations. Genotyping was performed with GeneSeekGenomic ProfilerPorcine 80KChip at Neogen. We collected a total of 1277 genotypes. Pedigree information was available in the PiggyBank database. To prepare genotyping data for analysis, we used macros within the statistical package SAS 9.4. PLINK 1.9 was used for quality control and the programme AlphaAssign for pedigree verification, which is part of the Alpha Genes Group Software. For confirmation results from AlphaAssign, we also checked genomic relationship coefficients using identical by descent function in PLINK 1.9. The pedigree verification and parentage assignment are performed in three steps. First, we associated the parents with all genotyped animals based on pedigree data in the database. For 1185 animals, both parents are known. For 92 genotyped animals, we do not have information about the parents in breeding documentation. Animals of unknown origin are animals without an ear tag or animals which do not have breed characteristics. In the second

step, we checked if we had parents' genotypes for those animals. The parentage of an animal can only be confirmed if the genotypes of the offspring and both parents match. Currently, we have 485 genotype triplets (animal, sire, and dam). In addition, for 365 animals, the genotype of one parent is known, so the parentage can only be partially confirmed. We do not have genotyped parents for 427 animals which belong to the older generations, and their parents were already culled. In the third step, we tried to find potential parents for the animals whose parentage was rejected, or there was no information about the parents in the documentation. A potential parent can only be confirmed if the animal's birth date matches the mother's reproductive data. We were able to confirm both parents in 358 animals, representing 77.5% of all verified pigs. Parentage for 30 animals was rejected completely (6 %). We found 36 animals with rejected boars and 43 animals with rejected dams. Out of 176 animals with information about sire genotype only, 91 % of sire was confirmed. Furthermore, for 89 animals with genomic information of dam only, 84.5 % of all dam was confirmed. The existence and development of the KP population require careful planning of animal mating to avoid related mating as much as possible and preserve the population's genetic diversity. Mating plans and other tools are only possible with correct parentage information. Therefore, we will continue genotyping and pedigree verification. KP breeders are informed about their results and the importance of pedigree correctness.

Keywords: Krškopolje pig, pedigree verification, genomic data

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Can we get rid of autozygosity in the crossbred pig?

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The high levels of autozygosity, small population size, and increased rates of inbreeding have raised concerns about the sustainability of conservation efforts in local European pig breeds. These unfavorable conditions raised the possibility of exploiting the use of crossbreeding as a means to contrast the loss of genetic diversity. Indeed, optimizing strategies for crossbreeding can help to expand the conservation management of farm animals by permitting a careful adaptive process with a long-term perspective. This study included 1133 individuals belonging to 23 pig breeds: 20 European indigenous breeds (n = 978) reared in 9 countries (Croatia: Black Slavonian, Turopolje; France: Basque, Gascon; Germany: Schwabisch-Hällisches Schwein; Italy: Apulo Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano, Sarda; Lithuania: Indigenous Wattle, White Old Type; Portugal: Alentejana, Bísara; Serbia: Moravka, Swallow-Bellied Mangalitsa; Slovenia: Krškopolje pig; Spain: Iberian, Majorcan Black), and three cosmopolitan breeds including Duroc, Landrace and Large White. All individuals were genotyped with the GeneSeek Genomic Profiler (GGP) 70 K HD porcine chip containing 68,516 SNPs. Twenty mating pairs drawn from each combination of breeds (in pure-breeding and crossbreeding) were simulated after phasing genotypes with the Beagle software (v.5.4). Runs

of homozygosity (ROH) were detected using the DetectRUNS R package. The distribution of ROH for each offspring was evaluated in order to analyse potential differences in the landscape of autozygosity between purebred and crossbred animals. The genomic inbreeding (F_{ROH}) was higher in purebred animals, as expected, especially in Mora Romagnola, Turopolje and Basque (average $F_{ROH} \geq 0.3$). Some interesting results were the high proportion of autozygosity observed in the following crosses: Alentejana and Iberian, Lithuanian local breeds and Large White, Basque and Gascon, and Mora Romagnola and Duroc ($F_{ROH} \geq 0.1$). These results suggest a close relationship among these parent breeds. The Large White and Landrace crossbreds did not show alarming F_{ROH} levels anyway, mating between these breeds and local breeds were characterized by high variability. All the other crossbreeding led to low inbreeding levels ($F_{ROH} \leq 0.1$) in the simulated populations, suggesting a potential use in future strategies.

Keywords: Crossbreeding, local pig breeds, homozygosity, conservation, simulation study.

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Association results in different Iberian pig populations between SNPs and phenotypes related to meat quality traits

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Meat quality traits are suitable to be included in breeding programs aimed at obtaining traditional products for specialized gastronomic markets. Furthermore, these traits are technically complex to measure and, as usual, are determined after slaughter, therefore selection is less efficient than other traits. For this reason, use of molecular markers for selection is recommended. Our group have been working for several years on the design and application of specific SNP panels for meat quality traits in Iberian pigs. A first panel of 64 SNPs, designed for intramuscular fat (IMF), was genotyped in 940 pigs from a Montanera (open free range based on pasture and acorn) commercial farm. Another panel of 32 SNPs, focusing on color, texture and water loss traits was genotyped in 950 pigs from the previous herd, in addition to 710 pigs from other different farms. Finally, a third panel of 32 SNP was built based on the results obtained from these designs, which included SNPs markers analyzed in the TREASURE project that could be used for a racial diagnosis of purebred Iberian pigs versus crossbred Duroc pigs. This third panel is the one that is already used routinely in the different Iberian pig populations involved in the studies and projects of our group. The association analyses were performed with different mixed models

that included systematic and environmental effects and covariates, specific in each case, in addition to the genetic effect with the kinship matrix. The allelic substitution effect was included as a fixed effect, taking values of 1 or –1 when the animal was homozygous or 0 when it was heterozygous. A multiple correction test was applied and those associations with a q-value less than 0.05 were considered significant. The most interesting results were the significant association between SNPs mapped in *ADIPOQ*, *ELOVL6*, *FASN*, *AMPD3* and *EGR2* genes with IMF and with the fatty acid profile of backfat, as well as between SNPs mapped in *PRKAG3*, *CAPN1*, *CAPNS1*, *CASP3*, *CTSL*, *ADIPOQ*, *ELOVL6*, *MYOD*, *MYH3* genes and traits related to color, texture or water loss. However, there have been several concerns when selecting the definitive SNPs to be used in Iberian pig breeding programs. First, the allelic frequencies are often extremes, since these local populations are usually closed, though in our populations most polymorphisms are segregating. Secondly, the study of the possible negative response with the carcass yield traits must be taken into account, especially with the percentage of hams, shoulders and loins. The high number of crossbred pigs with Duroc at a commercial level mainly justified by the higher yield of lean meat than the Iberian, forces us to be very cautious when selecting genetic markers. An improvement in meat quality accompanied by a reduction in carcass yield would mean a pure Iberian pig even less competitive than crossbred with Duroc. Finally, the heterogeneity of the breed makes it advisable to carry out prior specific studies before applying breeding programs to specific populations.

Keywords: Breeding programs, meat quality, Iberian pig populations, genetic markers

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Monitoring major gene polymorphisms in Italian pig breeds

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Morphological and productive traits are considered quantitative traits that are affected by many genes, a few of which can explain a quite relevant fraction of the genetic variability for the corresponding phenotypes. In pigs, several major genes affect breed-specific traits or traits that have been targeted by the domestication and selection processes. The monitoring of the variability at these major genes can be useful to evaluate if and how selection pressure generated by running breeding programs and genetic drift and bottleneck effects are driving allele frequency changes towards the fixation of the favorable alleles in the relevant breeds. In this study, we selected seven major genes that affect i) coat colour and colour patterns (*MC1R* and *KIT*), ii) growth rate and lean meat/fat deposition (*IGF2*), iii) vertebral and teat number (*NR6A1* and *VRTN*), iv) meat quality (*RYR1*) and v) hairless phenotype (*FOXN3*) and genotyped them in relevant pig breeds where a breeding or a conservation program has been designed or is considering to take into account the variability in these genes for the same purpose. A total of 752 boars and sows belonging to two cosmopolitan pig breeds (Italian Large White and Italian Duroc) and five autochthonous pig breeds (Apulo-Calabrese, Casertana, Cinta Senese, Mora Romagnola and Nero Siciliano), sampled in 2021, were included in this monitoring analysis. Allele and genotype frequencies in the different breeds provided information on the outcome of the running programmes and on the needs, in some cases, to re-address conservation and selection strategies. For example, as the favourable allele at the *VRTN* gene, associated with

increased number of vertebrae and teats, was the most frequent one in Italian Large White (0.56) and Italian Duroc (0.52), it is possible to envisage a good potential of improvement by selecting at this gene. The fixation of the *IGF2* g.3072A allele (associated with higher muscle mass deposition and growth rate) in the Italian Duroc breed is positive, considering the imprinted effects of this allele in crossbreeding programs. In Cinta Senese breed, the presence of the *KIT* g.43597545T allele (0.06) that when in heterozygous state does not produce the belted phenotype, calls for a culling action of the few heterozygous pigs in this population. A similar culling activity should be applied in the Nero Siciliano breed, where the relatively high frequency (0.15) of the defective *RYR1* c.1843T allele, causing the pale-soft-exudative defect of the meat, can increase the problem of poor meat quality. In conclusion, the monitoring strategy designed by genotyping a few targeted major genes in some Italian pig breeds should be further expanded to obtain additional genotyping information for other genes, which might be helpful to refine conservation and selection programs in these pig genetic resources.

Keywords: Autochthonous pig, candidate gene, SNP, *Sus scrofa*.

Acknowledgement

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SESSION 2

PRODUCTION SYSTEMS, FEEDING AND ENVIRONMENT

MAIN LECTURES

Growth potential of Krškopolje pigs in different production systems

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The interest for Slovenian autochthonous pig breed– the Krškopolje pig is increasing, while knowledge about its nutritional needs is limited. Because Krškopolje pigs are reared in diverse production systems, from extensive backyard to intensive indoor system, their phenotype is more complex. In order to estimate their nutritional needs, trials were conducted to obtain the data on body weight, backfat thickness, cumulative feed intake, feed (ingredients and chemical) composition in traditional (TRAD; indoor housing in pens with full floor, straw bedding, and access to an outdoor area; n=6), indoor (IND; partly slatted floors; n=14), and outdoor (OUT; n=12) system. Data were used to generate animal profiles with InraPorc® software following the methodology described in Brossard *et al.* (2019). Growth performance on *ad libitum* feeding regime was simulated for the entire growing-fattening period typical for Krškopolje pigs (30 to 160 kg body weight). As expected, the results of performance simulation with different animal profiles showed the highest growth potential of Krškopolje pigs in the IND system (average daily gain (ADG) of 734 g, with an average daily NE intake (NE intake) of 31.43 MJ NE). Compared to IND system, pigs in TRAD and OUT systems had approximately 8% and 14 % lower ADG, respectively, at 15% higher and 4% lower NE intake, respectively. Due to approximately 19% and 23% lower average daily protein deposition they exhibited approximately 16% and 19% lower SID lysine requirements per day compared to IND system, respectively. As expected for fatty type pigs, the proportion of NE intake retained as lipid was high, regardless of the rearing system (40.2%, 39.7 and 43.6% in IND, TRAD and OUT, respectively), and NE intake retained as protein quite low (7.6%, 5.2% and 6.1% in IND, TRAD, and OUT, respectively). In conclusion, the

present study confirms that the production system in which Krškopolje pigs are kept influences their growth potential and nutritional needs. To formulate feeding recommendations for different production systems, further simulations for different growth and fattening phases, and with different animal profiles are needed.

Keywords: fatty pig, modelling, protein deposition, nutritional requirements, production system

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Environmental impact of traditional low-input pig production systems

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The main environmental impacts of pig production are associated to water, soil, and air pollution. Water pollution is due to improper disposal of pig manure and leaching of N and P compounds from agricultural land into groundwater or direct runoff of organic effluents and pathogens into surface waters. This disrupts ecosystems through eutrophication and acidification, and limits the use of water for human needs. Soil pollution occurs due to excessive accumulation of phosphorus or trace elements (Cu, Zn). Air pollution consists of the emission of ammonia and greenhouse gases (GHG - carbon dioxide (CO_2), methane and nitrous oxide) as well as odour spreading. Ammonia and other nitrogen compounds and methane emissions come from livestock facilities and from poorly managed manure storage and spreading. Emissions of other GHG, mainly CO_2, come from energy consumption (fuel and electricity) for feed production and pig facilities maintenance, as well as from the transport of the necessary inputs, including the main agricultural products (maize, barley, soybeans) and the raw materials for their production (fertilisers and pesticides). Therefore, both air and water pollution to be considered when evaluating the environmental impact of pig production system. Low-input pig production systems are characterised by low investments in facilities and labour, keeping pigs in smaller herds with many pigs outside, the use of indigenous local breeds and often non-standard on-farm management practises (here comprised feeding) adapted to local conditions. Feeding is based on locally produced cereals (maize, barley, and wheat), agricultural by-products and other plant material from pastures (grass, acorns, and chestnuts). Local feed supply chain and

using indigenous breeds well adapted to local climate conditions determines specificity of traditional pig production system. A small herd kept in free-range conditions reduces the amount of manure that needs to be managed during storage and spreading and consequently reduces methane and N emissions during manure manipulation. Animals kept on pastures deposit manure (excrements) directly on the ground. The rapid absorption of urine and liquid faecal components after defecation reduces the possibility of surface leaching of N. Most of the N is absorbed very quickly by the pasture vegetation and does not reach deeper soil layers. In addition, the lower requirement for feed protein in indigenous breeds can potentially reduce N content in their manure. Feeding pigs with locally produced feed and grazing contributes to lower GHG emissions due to the lower energy consumption for feed production and the reduced use of agricultural inputs. In addition, traditional free-range farming of indigenous pig breeds is associated with lower facility maintenance, lower fuel, electricity and water consumption, and less transport of the required inputs, leading to a significant reduction in GHG emissions. On the other hand, indigenous breeds in low-input production systems show high variation in reproductive and fattening performance. Low fertility and high fat deposition in the carcass have a negative impact on feed conversion, which is one of the most important determinants of the environmental impact of pig production.

Keywords: Environmental impact, pig, production system, local breed, low input

SESSION 2

PRODUCTION SYSTEMS, FEEDING AND ENVIRONMENT

ORAL COMMUNICATIONS

Production systems and feeding of local breeds of pigs in Republic of Serbia

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In recent years, the popularity of local pig breeds has increased in the Republic of Serbia. Local pig breeds such as Mangalitza, Moravka and Resavka, have again found their place in rural households and in the overall biodiversity of Serbia. Their increasing number led to a diversity of production systems and feeding types. They ranged from fully intensive systems with commercial mixtures to completely extensive without supplementing any additional feed except what the animals find on the ground. Aim of this study was to determine the differentiation of these systems by region and to examine the differences between them. We classified all these production systems into six categories: intensive systems using commercial mixtures, intensive systems using cereals, vegetables and fruits as the main feed, semi-intensive systems using commercial mixtures, semi-intensive systems using cereals, vegetables and fruits as the main feed, extensive systems using cereals, vegetables and fruits as supplemental feed and completely extensive systems. We studied the main advantages and disadvantages of these systems, including economic factors. The results showed extreme differences, especially in animal size, investment, and economic aspects. Contrary to wide opinion the lowest investments were on farms with fully intensive systems because they used old concrete buildings that were used for commercial breeds in the past. On the other hand, breeders with full extensive systems have made the most investments, considering the cost of the land. The size of the animals and the type of feed used were in positive correlation. Animals fed commercial mixtures were almost

double in size and weight than those raised in fully extensive way. The cost of feed dictates which feeds are used in the animal's diet, especially considering grains, vegetables, and fruits. Although the investment in fully intensive systems is lower, the health aspect and welfare of the animals in these systems should be studied. Besides the health aspect, it is questionable how these breeding systems affect the quality of the final products of these local breeds. Furthermore, it will be interesting to investigate how each of these breeds performs in all these production systems. Also, further studies will investigate how these types of production systems affect the value of the final pig products on the market.

Keywords: Pig, local breed, Moravka, Resavka, Mangalitza

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Banija spotted pig – from science to practise

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The aim of this paper is to present the main research activities performed in the Banija spotted (BS) pig and to point out some practical challenges for future work related to breeding activities. After the official recognition of the BS pig at the end of 2018, several research areas have been developed related to this local pig breed. These activities were mainly an extension of earlier completed national projects with the aim of official breed recognition, increasing the population, and obtaining information on the main production traits of the BS pig. The genetic diversity of the BS pig has been studied in detail using microsatellite and pedigree data. The BS pig populations exhibit great genetic diversity and are genetically distinct from neighbouring geographically proximate pig populations. Based on microsatellite and pedigree data, two subpopulations and a relatively high inbreeding rate per generation can be identified. As population size and genetic diversity continue to increase, optimal contribution selection is proposed as the method of choice. Another research area refers to the reproductive characteristics of BS pigs, which are among the most important traits from an economic point of view. The BS pig breed is characterised by moderate fertility with a litter size of approximately 7 to 8 piglets born alive. The variability of litter size between farms is relatively high and could be partly related to the different farm rearing systems and management practises. The growth traits of the BS pig are satisfactory with average daily gains between 300 and 500 g, depending on the production system and feeding level. Fattening pigs reach final weights of 150 kg or more at 16 months of age. Slaughter traits of BS fattening pigs show high processing value of the breed. Analysis of meat quality traits showed favourable

values for meat pH, colour, and water-holding capacity, with no noticeable defects. The current state of BS pig farming was recently surveyed through visits to pig farmers as part of the GEroNIMO project. It was found that the activities of the breeding organisation were directly affected by the COVID crisis and the 2020 Banija earthquake. A major problem for breeders is the lack of a slaughterhouse or meat industry interested in processing BS pork. The breeding area of BS pig has expanded to neighbouring districts, which is positive from the point of view of biosecurity and population expansion. In addition, some younger farmers are starting to raise BS pigs, which is very promising for future work and development on this breed.

Keywords: pigs, Banija spotted pig, production traits, breeding

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Interactions between three Iberian pig strains and two feeding systems for changes in blood metabolic parameters during finishing

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This study compares the changes in blood metabolic parameters of three Iberian pig strains finished in two systems. Sixty-five castrated pig males of three different strains: 20 *Lampiño* (L), 23 *Torbiscal* (T) and 22 *Retinto* (R), finished in two systems: acorn-feeding, extensive *Montanera* (Mt; n=34; 11 (L), 12 (T) and 11 (R)) and semi-intensive *Cebo de Campo* (CC; n=31; 9 (L), 11 (T) and 11 (R) were used in this study. Pigs were raised together with the same feeding management up to the age of 13-14 months, then, they were separated into the two finishing groups. Pigs started the finishing period at 100, 104 and 109 kg of body weight (BW) and were slaughtered at 155, 164 and 169 kg of BW for L, R and T, respectively. At the beginning and at the end of the finishing period, 10-ml blood samples were collected from each animal and, after centrifugation, plasma samples were stored at -20ºC for further analyses. Plasma levels of urea, triglycerides, cholesterol, lactate, glucose and fructosamine were determined by an automated system (Saturno-300 Plus Analyzer; Crony Instruments). Differences (-d) in plasma levels from the beginning to the end of the finishing period (urea-d, triglycerides-d, cholesterol-d, lactate-d, glucose-d and fructosamine-d) were calculated. Data were analysed by GLM procedures with a model including final BW or average daily gain (ADG) as covariates, genotype, feeding system and the interaction genotype x feeding system. At the beginning of the

finishing period, urea and triglyceride levels were significantly higher in L vs. R and T strains, and fructosamine levels were higher in T vs. R. At the end of the finishing, there were no differences in urea, lactate, fructosamine, and cholesterol levels among the genotypes, but the levels of triglycerides and glucose were significantly higher in the R strain than in the other two strains. As for the finishing system effect, the levels of urea, triglycerides, lactate and glucose were higher for animals fed in CC than for those fed in Mt. In relation to level of change of the biochemical parameters studied during finishing, there was a trend for interaction for urea-d (P=0.08) (with the greatest difference between systems occurring for L); also for triglycerides-d, (P=0.07) (significantly greater in CC than in Mt, being these changes greater in R than in L or T); and finally for fructosamine-d (P=0.07) (significantly greater during Mt *vs* CC for R but was similar between systems for L and T). For glucose-d, there was a significant interaction (P<0.005), since the increase in glucose level during finishing was greater in CC than in Mt only for R and T but not for L. In conclusion, blood parameter changes were affected by genotype, feeding system and their interaction. For example, protein metabolism was more affected by feeding system in *Lampiño*, in which glucose metabolism was not affected by feeding system. Finally, lipid metabolism was much more affected by the feeding system in *Retinto* than in the other two strains.

Keywords: Genotypes, biochemical parameters, fattening system, extensive system, swine

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Effects of dietary tannins on parotid gland growth and saliva composition of Iberian pigs

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The ingestion of tannins associated with acorn consumption may lead to changes in saliva flux and composition affecting salivary gland growth. The objective of the present work was to investigate the effects of length of tannins consumption on parotid gland development and composition and on saliva amino acid (AA) composition. Eighteen purebred Iberian pigs of 105 ± 1.5 kg body weight (BW) were randomly allocated to three experimental groups (n=6) fed either a control diet (C; based on barley (91%) and soybean meal (5%)) for 36 days, or an enriched tannin diet (barley (72%), olive pulp (15%) and carob bean (10%)) for 16 (T1) or 44 days (T2), respectively. Feed and water were provided *ad libitum*. Feed consumption was recorded daily and BW was registered weekly. At the end of the experiment, pigs were slaughtered by electrical stunning and exsanguination. The day before slaughter, saliva samples were collected -using small chewed sponges- from all experimental pigs and stored (-80º C) until analysis. Immediately after slaughter, organs and main carcass components were weighed and parotid glands were dissected, trimmed of fat and connective tissue, weighed and stored (-20º C) until analysis. Experimental diets were analysed for nutrient composition. Parotid glands and saliva were freeze-dried and analysed for total

nitrogen content and AA composition. Dietary treatment effects were evaluated using the GLM procedure of SAS. Significant differences between treatment means were determined using Tukey t-tests. Results were considered significant at $P < 0.05$. Relative kidney weights (g/kg empty BW) increased by 20% in T1 pigs compared to C pigs, although after longer tannin consumption there were no differences between C and T2 groups. Relative weights of stomach and spleen were 16% lower in T2 compared with C pigs with no differences between T1 and C groups ($P < 0.05$). Parotid glands relative size (g/kg empty BW) increased by 15% after 16 days of tannin consumption (T1 > C; $P < 0.05$), although no size differences were detected between C pigs and pigs that consumed the enriched tannin diet for 44 days (T2). Total protein content of parotid glands (g crude protein/100 g freeze-dried gland) was not different between treatments. There were small differences in AA composition of the gland (mg AA/g crude protein) between treatments and no effect on proline content ($P > 0.05$). However, proline content of saliva increased more than 100% in T1 pigs compared with C pigs, although T2 pigs showed intermediate values ($P < 0.05$). Increase in proline concentration of saliva could be related to an increase in proline-rich protein secretion in response to tannin ingestion. The use of parotid gland size as an approximate marker of tannin (and acorn) consumption needs further investigation, although it seems that in fattening Iberian pigs gland size is not affected after an initial adaptation period.

Keywords: Dietary tannins, Iberian pig, parotid glands, proline, saliva

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SESSION 3

ANIMAL HEALTH, PHYSIOLOGY, WELFARE AND REPRODUCTION

MAIN LECTURE

In vitro gene preservation of Hungarian Mangalica pig in the shadow of ASF

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African Swine Fever (ASF) affected the global as well as the European pig production, recently. Pathway of it has been investigated and documented well, started from East Africa via large curve in Eastern countries and approached to EU. Pig breeding and pork production suffered unforeseen economic disaster in many countries of significant pig sector. No exact data are available but about 8 to 10.5 million pigs were slaughtered in order to prevent its further spread. It was difficult to prevent spread of ASF to the West mainly due to human and wild boar vectors. Hitherto Hungary could avoid the touch of ASF in domestic pig population however wild boar population was contaminated to the line of river Danube dividing the country to two similar size parts. It is well known that international commercial restrictions are almost the same whether ASF presence was proven in wild boars or domestic pig population. Anyway bilateral agreement of seller and buyer countries can enlighten the trading of pork e.g. on the basis of so called regionalization. Despite huge economic disaster big international companies have relevant backup to replace the reduced population by using genetic resources at different geographical locations safe from ASF till the moment. Indigenous swine breeds of some countries and regions represent a special national and commercial value. If they disappear due to any pandemic disease or preventive culling the population can be hardly or cannot be replaced at all. Foreign examples underline the importance that apart from in vivo gene preservation strategic in vitro gene reserves are also

needed. Our experts clarified the present situation that Red, Black and Swallow-Bellied Mangalica as well as the endangered Hungarian Large White population can disappear if they are infected by ASF and cannot be renewed in the future. The University of Veterinary Medicine Budapest was appointed by the Ministry of Agriculture to collect a group of scientists and practical breeders in Hungary create an in vitro gene reserve of the mentioned Hungarian pig breeds in cooperation with the relevant breeding associations i.e. National Association of Mangalica Breeders and Hungarian Purebred Pig Breeders' Association. A total of 60 to 100 boars and 20 to 30 gilts have been included in the project of collecting and deep-freezing seeds, or early embryos. Approximately at mid time of the program more than 1500 straws of semen of 5 Mangalica and 18 HLW boars were deep frozen. Semen quality were controlled by CASA and only ejaculates with more than 80% motile sperm were used for deep freezing. Before starting the project, we supposed that most sensitive points would be the collection of semen from the selected boars of rare strains and the freezing ability of different ejaculates as well as the deep-freezing of the early embryos. Boar semen and embryo deep-freezing has been a known technique for a long time, however it should be adapted in a breed specific manner.

Keywords: Mangalica, ASF, gene preservation, semen, embryo

SESSION 3

ANIMAL HEALTH, PHYSIOLOGY, WELFARE AND REPRODUCTION

ORAL COMMUNICATIONS

Antibiotic-free hypothermic storage of Iberian boar semen: *in vitro* sperm function and bacterial load

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In pig farms, the fertilization is performed by artificial insemination (AI) using seminal doses (SD) preserved at 16 ± 1 ºC with commercial extenders in which routinely antibiotics (AB) are added. The inappropriate and indiscriminate use of AB contributes to the emergence of resistant bacteria. As an alternative to common SD preservation in the presence of AB, hypothermic storage at 5 ± 1 ºC in the absence of AB has been proposed. The objective of this study was to determine an appropriate methodology to optimize Iberian boar seminal doses conserved diluted at 5 ± 1ºC in an antibiotic-free extender. Semen from six Iberian boars extended in MRA® Antiox without AB was cooled from room temperature to 5 ± 1ºC using an equilibration time at 16 ± 1ºC of 6h (protocol 1) and 20h (protocol 2), and a cooling rate of 0,025ºC min^{-1} from 16 ± 1ºC to 5 ± 1ºC. Semen from the same Iberian boars extended in MRA® Antiox with AB and conserved at 16 ± 1ºC were used as control. SD were analyzed 4 and 6 days after refrigeration diluted at 16 ± 1ºC or 5 ± 1 ºC. SD preserved with protocol 2 maintain high motility and progressivity, membrane integrity, mitochondrial membrane potential and low ROS production without statistical differences with SD preserved at 16 ± 1ºC diluted in MRA® Antiox with AB, both at 4- and 6-days preservation. However, reducing equilibration time at 16 ± 1ºC (protocol 1) results in statically significant lower values of all sperm parameters. Bacterial load $<10^3$ CFU mL^{-1} was measured in all SD stored at 5 ± 1ºC in

AB-free extender and no differences were found with control SD stored at 16 ± 1ºC with AB. In conclusion, an AB-free preservation of Iberian boar SD protocol has been established at 5 ± 1ºC allowing Iberian porcine AI to be performed in the absence of antibiotics.

Keywords: Iberian pigs, seminal doses, antibiotics, hypothermic storage.

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Analyses of heat stress effects on the vagina microbiome of Iberian sows

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Heat stress (HS) is one of the consequences of climate warming since it could alter the physiology of animals, reducing female and male reproduction and increasing mortality at birth. Heat stress effects altering porcine microbiome have been reported. Iberian pig breed is autochthonous from the Southwest of the Iberian Peninsula, where they have been adapted to high temperatures and food and water shortage during summer. The aim of the present study was to analyse the microbiome profile in samples from vagina exudate from 21 Iberian sows at two time points: June (heat stress (HS); average temperature = 23.84ºC) and November (non-heat stress (NHS); average temperature = 9.84ºC). Exudate samples were collected using swabs from the vagina of sows during the oestrus, in two consecutive cycles. Microbiota DNA was extracted from 42 vagina exudate samples and 16S rRNA gene was sequenced with Illumina MiSeq. Reads were processed with qiime2 and amplicon sequence variant (ASV) were defined. SILVA reference database (release 138) was used for taxonomic assignment. Microbial composition, alpha and beta diversities were determined using phyloseq. To carry out the analyses of beta diversities, compositional data analyses were carried out with zCompositions and easyCODA. Differential abundance analyses were performed with EdgeR (not considering compositionality) and Linda (considering compositionality) R packages. After filtering, chimera exclusion and removing reads not assigned to

Bacteria kingdom, a total of 577,258 reads and 4,077 ASVs remained, being 88.37% of the reads assigned to phylum level. A total of 19 phyla were identified, being Firmicutes and Proteobacteria the most abundant. Heat stress effects on alpha diversities were analyzed with repeated measures ANOVA test, which showed no differences between HS and NHS periods in the indices estimated (observed richness, Shannon, Simpson, Fisher and ACE). Low clustering and distance between centroids were also observed in compositional PCA for beta diversity. However, PERMANOVA revealed significant differences in beta diversities between HS and NHS samples at ASV, family and genus level. While no ASV was observed as differentially abundant between HS and NHS periods with EdgeR software, Linda showed a total of 77 ASVs differentially abundant ($|log2FoldChange| > 0.58$ & $p\text{-value} < 0.05$) between both collecting periods. Nevertheless, none of them remained significant when multiple testing correction was applied. A total of 59 ASVs were overabundant in samples collected at HS period and 18 were overabundant at NHS. It is worth to mention that the genus *Bacteroidales RF16* decreased during HS period, other authors also observed this reduction in fecal microbiota of primiparous pigs during late gestation. According to the results obtained, the microbiome of vagina is not very different between HS and NHS periods. In microbiome analyses, the obtained results are sometimes dependent on the filtering and corrections carried out, because of that, some of these results can be considered as preliminary and further analyses have to be made.

Keywords: heat stress, vagina microbiome, prolificacy, Iberian pigs

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Leg weakness, osteomalacia and hypocalcemia in weaned Black Slavonian piglet: A case report

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In recent years, interest in native breeds playing an important role in the conservation of agricultural biodiversity and traditional production systems has increased. However, these production systems, despite of providing greater behavioural freedom for animals, may jeopardise their health and welfare, especially from the nutritional aspect. This case report describes the reason for culling the weaned Black Slavonian piglet. The piglet was five-month-old, from the first parity sow and raised in an outdoor production system. After weaning, retardation in the piglet growth, poor leg conformation with alterations in bone length and diameter, and lameness were visible. Macroscopic post-mortem analysis revealed leg weakness, osteomalacia, and hypocalcemia. Progressive and irreversible deformities in bone structure and joints were observed along with a low level of bone mineralization and softening indicating deficiency of calcium, phosphorus, and vitamin D. Lameness is generally the second most common cause of pigs being culled. Osteomalacia as a condition is more common in commercial production systems in first litter gilts in particular due to still undeveloped skeleton and high demands on calcium for milk production. This case report finding could

also be attributed to non-adequate management mainly inappropriate nutrition, but also to other factors such as infectious and parasitic diseases, stress, aggression, lesions, toxic compounds, climate and housing conditions, etc. Therefore, more attention should be paid to good stockmanship, adequate management, prevention, and health control in native pig breeds kept in outdoor production systems to raise healthy, strong, and vital animals. A crucial role of the stockperson is to assess the interaction between the pig, its age, and productive cycle in relation to the feed quality, content, and intake. The role of the management is not only to ameliorate potential diseases in the herd but also for the pig to maximize its biological potential. Non-invasive technique such as urine chemistry analysis is a helpful and simple method to investigate and prevent nutrition-related problems.

Keywords: Black Slavonian pig, leg weakness, osteomalacia, hypocalcemia

SESSION 4

MEAT AND PRODUCT QUALITY, INNOVATIONS AND TRACEABILITY

MAIN LECTURES

Underlying mechanisms of lipid deposition in local and modern pig breeds

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While intensive selection resulted in accelerated growth rate and better lean meat deposition in modern pig breeds, local breeds still retain lower performance capabilities. On the other hand, they exert better adaptation to specific local environments, including local feed sources, environmental food shortages and seasonal food availability. Along with lower growth potential, they have the ability of higher fat deposition, which reflects their particular lipid metabolism properties. Although genetic, biochemical and physiological mechanisms, that result in the differences between local and modern breeds are still not fully explicated, many conclusions can be drawn, based on available research. Besides higher fat accumulation, the lipids in local breeds contain more saturated and in particular more monounsaturated fatty acids (mostly oleic acid). Specific fatty acid composition may be associated to higher activity of lipogenic enzymes, higher and more persistent desaturation ability and lower potential for lipolysis in either subcutaneous or intramuscular fat depot. Higher adiposity of local breeds is reflected on histological level, as better adipocyte hyperplastic and hypertrophic abilities are leading to higher adipocyte size. In addition, hormone-related specificities were also indicated in some local breeds, including leptin resistance. As to the transcriptomic and proteomic studies, several gene groups are uncovered that differ between the breed types. In subcutaneous fat tissue, these include downregulation of genes involved in mitochondrial energy metabolism and development of the extracellular matrix and upregulation of genes and proteins associated to fatty acid transportation, lipogenesis,

lipid desaturation, insulin signalling/response and immune response in local compared to modern pig breeds. In intramuscular fat, upregulation of additional genes related to adipogenesis, fatty acid mobilization and lipolysis were indicated in local breeds. The research aiming at detecting selection signatures characteristic to the European local breeds (leading to adaptation to specific local environments) pointed out several interesting results. These include genes related to adipocyte lipid degradation, immune response and taste receptors in addition to several other genes of lipid metabolism. A recent study performed a selection scan with link to phenotype and recognized several genomic regions, which could affect fatty phenotype in European local pig breeds, with individual candidate genes involved in energy balance, adipose tissue development and inhibition of protein synthesis in response to stress.

Keywords: pig, local breeds, fat depots, lipogenic enzymes, fat tissue properties, metabolism, gene expression

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Different understandings of pork quality - a historical overview

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This paper addresses the various interpretations of pork quality and its changes with the development of scientific methods, pork industry requirements, and changing consumer opinions on pork and pork products. Pork quality has been under the scrutiny of scientific studies for almost three quarters of a century. Various interpretations of the term have led to considerable confusion not only among pig producers and processors, but even more often among the consumers. Since the early days of meat quality characterisation and evaluation, the relationship between the rate of pH change *post-mortem*, colour and water holding capacity of the pig muscle has been considered as the main determinant of the meat status of pigs. In the meanwhile, the number of meat traits and the methods to measure them have increased considerably with the continuous development of scientific methods and equipment. The multidimensionality of meat quality is reflected in the interaction of genetic make-up of the pigs, rearing conditions, manipulations during the transport and slaughter, and post mortal changes in the transformation of muscle into meat. This makes quality of pork a very complex phenotypic trait. The aim of this paper is to present the historical development of the concept of pork quality, from the first observations of differences in muscle structure characterised by water-holding capacity and colour to the development of biomarkers as regulators of animal phenotypes.

Keywords: Pig, meat, quality traits

SESSION 4

MEAT AND PRODUCT QUALITY, INNOVATIONS AND TRACEABILITY

ORAL COMMUNICATIONS

Loin and fat quality of Portuguese local Alentejano and Bísaro pigs and their crosses

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Alentejano (AL) and Bísaro (BI) Portuguese local pig breeds cohabited in Ribatejo region for some time. Their crosses (RI pigs) and the products obtained, although appreciated, disappeared in the 1950's without being scientifically evaluated. To assess the meat quality of RI pigs, castrated males from AL, BI, ALxBI and BIxAL genotypes were studied within the framework of the TREASURE project. Raised in a traditional outdoor system and fed commercial diets *ad libitum*, pigs were slaughtered at ~65 (Trial 1, n=10 for each genotype) and ~150 kg live weight (LW) (Trial 2, n=9 for each genotype), and *Longissimus lumborum* (LL) and dorsal subcutaneous fat (DSF) physical-chemical traits were analysed. When compared to BI, AL pigs presented LL with lower moisture (trial 1 - 73.1 *vs* 74.7; trial 2 - 71.0 *vs* 72.1 g/100g; P<0.05), and higher IMF (trial 1 - 6.7 *vs* 5.5; trial 2 - 6.9 *vs* 6.0 g/100g; P<0.05) content. LL samples from AL also had higher myoglobin (trial 1 - 0.76 *vs* 0.54, P<0.007; trial 2 - 0.86 *vs* 0.45 mg/g, P<0.05) and lower total collagen (trial 1 - 13.9 *vs* 17.1, P<0.05; trial 2 - 13.1 *vs* 16.7 mg/g DM, P<0.01) content. As to RI pigs, compared to the pure genotypes, they presented intermediate values of protein, IMF and total collagen. However, LL content of moisture, total pigments and tenderness were similar to the ones from AL. When compared to BI, LL from AL pigs had similar proportions of saturated

(SFA) (trial 1 - 35.8 *vs* 36.8, P=0.126; trial 2 - 34.2 *vs* 35.8 g/100g, P=0.094), higher proportions of monounsaturated (MUFA) (trial 1 - 48.0 *vs* 40.2, P<0.001; trial 2 - 48.4 *vs* 43.1 g/100g, P<0.05), and lower proportions of polyunsaturated fatty acids (PUFA) (trial 1 - 16.2 *vs* 23.0, P<0.001; trial 2 - 17.4 *vs* 21.1 g/100g, NS). RI pigs presented intermediate proportions of FA between the fatter (AL) and the leaner (BI) genotypes. Results of RI pigs, when compared to those of AL indicate their potential for the production of loins with the similar rich colour and tenderness, and with an intermediate FA profiles between AL and BI genotypes. As for DSF, when compared to BI pigs, AL presented lower moisture (trial 1 - 7.3 *vs* 11.0, P<0.0001; trial 2 - 5.1 *vs* 5.8 g/100g, NS) and higher total lipids content (trial 1 - 85.0 *vs* 71.5, P<0.0001; trial 2 - 88.9 *vs* 83.7 g/100g, P<0.05). As for FA proportions, DSF from AL presented similar proportions of SFA (trial 1 - 38.8 *vs* 40.2; trial 2 - 39.8 *vs* 41.1 g/100g; NS), higher proportions of MUFA (trial 1 – 49.0 *vs* 44.3, P<0.0001; trial 2 – 49.9 *vs* 46.2 g/100g, P<0.002), and lower proportions of PUFA (trial 1 – 12.2 *vs* 15.6; trial 2 – 10.4 *vs* 12.7 g/100g; P<0.001) when compared to BI. Again, the RI pigs had an intermediate proportion of FA between AL and BI genotypes. In both tissues, oleic acid (C18:1 n9) was the most abundant FA in all genotypes, followed by palmitic acid (C16:0). RI pigs could therefore be an alternative to using other breeds in commercial crosses with the pure genotypes when the production of pure breeds is not economically beneficial.

Keywords: Local pigs, Alentejano pig, Bísaro pig, Ribatejano pig, loin and fat quality

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Biochemical and gene expression differences associated with higher fat deposition in Krškopolje pigs – comparison with lean crossbred pigs

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In Slovenia, there is only one autochtonous local pig breed, the Krškopolje pig. The interest for this endangered breed is lately increasing. One of the important reasons for this is a better quality of meat and fat, which enables the production of high-quality products. Krškopolje pig is also well adapted to local feeding resources and extensive rearing systems. This breed has not yet been studied with regard to the mechanisms explaining much greater fat deposition compared to modern lean pigs. Therefore, the objective of the present study was to determine biochemical and gene expression differences associated with fat deposition. Six Krškopolje and six modern hybrid pigs (progeny of Landrace x Large White sows and Duroc boars) were reared in equal environmental conditions and were fed the same diet. At an average body weight of 165.6 ± 8.2 kg (mean ± SE), the animals were slaughtered in a commercial abattoir according to standard slaughter procedures. Carcass traits were measured and intramuscular fat was determined in the *Longissimus dorsi* muscle. Fatty acid composition, the activity of lipogenic enzymes (malic enzyme, glucose-6-phosphate dehydrogenase, citrate cleavage enzyme), and expression of genes involved in lipid metabolism (using qPCR) were determined in backfat samples taken at the level of the last rib. As expected, Krškopolje pigs exhibited higher backfat thicknesses at different anatomical locations (i.e. last rib, at the withers, and above the *Gluteus medius* muscle; $P<0.001$), whereas the intramuscular fat content of the *Longissimus dorsi* muscle did not differ between groups ($P>0.05$). Malic enzyme and glucose-6-phosphate dehydrogenase

activities were 2.59- and 1.98-fold lower, respectively, in the Krškopolje pigs than lean crossbred pigs (P<0.01). However, no significant differences were found for the citrate cleavage enzyme (P>0.05). Compared to the lean crossbred pigs, the backfat of Krškopolje pigs contained a higher content of monounsaturated (MUFA) and a lower content of polyunsaturated fatty acids (P<0.01) while the content of saturated fatty acids did not differ (P>0.05). Consistent with the higher MUFA content, overexpression of the stearoyl-CoA desaturase gene was also detected in Krškopolje pigs when compared to the lean crossbred pigs (P<0.01). In addition, higher expression of genes involved in lipogenesis (i.e. *ACACA*, *FASN*, *PPARγ*) or energy homeostasis (i.e. *LEP*) was observed in Krškopolje pigs than in modern crossbred pigs (P<0.05). In conclusion, Krškopolje pigs exhibited higher fat deposition associated with higher MUFA content, and higher expression of genes involved in lipogenesis than in lean crossbred pigs. Controversially, the activity of lipogenic enzymes was still found lower in Krškopolje pigs.

Keywords: local pig breed, lipid deposition, subcutaneous adipose tissue, fatty acid composition, lipogenic enzyme activity, gene expression

Acknowledgement

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Implementation of traceability in Krškopolje pig

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This research aims to implement methods of traceability and verification of animals or meat and establish a traceability system to assure the source of meat is Krškopolje pig (KP). To provide the consumer with a meat product with declared characteristics and origin, it is necessary to introduce protocols to ensure and verify the traceability of pigs and meat. Therefore, unique identification of animals, meat or products of animal origin has to be maintained through various steps of the whole food chain. At first, we checked the zootechnical documentation required by Breeding Program for the autochthonous Krškopolje pig and legislation. The information flow was depicted and equipped with possible weak points based on possible recording mistakes. Traceability of pigs is assured by covering pig lifespan from birth to slaughter using routine zootechnical documentation. All pigs are marked with unique identification numbers (ID) and origin farm numbers. Furthermore, traceability after slaughter is guaranteed by the documentation required at the slaughterhouse. To verify the source of meat, we used genetic information from GeneSeekGenomic ProfilerPorcine 80KChip at Neogen. In total, 1346 samples of tissue or meat products that originated from various sources and breeds were genotyped. After quality control performed by program PLINK 1.9, 40 002 markers, and 1277 samples were retained for the analysis, including 19 different samples of meat or meat products that have been branded as KP meat and originated from seven producers. Software AlphaAssign was used to search for potential

parents. We expected that potential parents were all breeding animals in the breeding program with at least one known farrowing with mating recorded. Relationship coefficients were computed using the identity by descent function in the PLINK program. The expected relationship coefficient of an individual with a parent is 0.5, and with grandparents, 0.25. Full sibling coefficients are variable, with a high probability between 0.4 and 0.6. For the individuals found to be related to the samples, we checked if the obtained genomic information was consistent with records in herdbook information. Traceability of KP is obtained from reproduction data such as mating, farrowing, weaning, marking and trading. All pigs must be marked on the right ear with the two-letter country code "SI" and the last six digits of the G-MID of the farm of birth. In addition, all KP pigs must be marked on the farm with a unique identification number on the ear tag before weaning or mixing piglets. Traceability is assured in the slaughterhouse, where each carcass or its part is marked with key information for declaration. Out of all samples, seven meat samples were identified as being originated entirely from the KP breed. Mostly, assigned parents had known mating dates and had genomically confirmed pedigrees. Only in one meat sample we could not match a mating of sire and dam, concluding that found sire with the relationship coefficient of 0.489 could be a full sibling to the pig that the meat originates. Three samples had partially confirmed origin with either of the parents written into the breeding book. In 4 meat samples, we suspect that there was a migration of a different breed in a second or third generation. We can partially confirm the origin of animals and meat with the current methods. However, research has been directed toward genomic breed verification.

Keywords: pigs, indigenous breeds, Krškopolje pig, genomic pedigree, traceability

Acknowledgement

The research was funded by an EIP project *Sledljivost porekla pri pasmi krškopoljski prašič* (3011/2018/11)

Sensory profile of Croatian dry-cured hams: PDO (Istarski Pršut) and PGI (Krčki, Dalmatinski and Drniški Pršut)

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The aim of this study was to characterize dry-cured hams from four different processing methods, with differences in primary leg treatment, salting and smoking phase. Many biochemical changes (lipolysis, proteolysis, oxidation reactions, Strecker degradation and Maillard reactions) take place during the manufacturing and ripening of dry-cured ham and contribute to flavour development. The samples used in this study were four types of Croatian dry-cured hams of which three with Protected Geographical Indications (PGI) (Krčki, Dalmatinski and Drniški pršut) and one with Protected Designation of Origin (PDO) (Istarski pršut). The research was carried out on 24 dry-cured hams, obtained by processing 24 pig legs following the different specifications. Processing of dry-cured hams was performed according to four protocols, which differ in the stages of smoking (smoked dry-cured ham: Dalmatinski and Drniški pršut) and salting with a mixture of spices (non-smoked dry-cured ham: Istarski and Krčki pršut). Sensory analysis was carried out by a trained panel (8 judges), selected and trained following the procedures of the ISO standard (ISO 11132: 2012). Furthermore, the precision and trueness of each expert assessor were determined by the repeatability index (Ri_a), and intermediate precision index (IPi_a), and homogeneity of panel CVR%. The following attributes were evaluated: color intensity of muscle and fat tissue, color uniformity, amount of intramuscular fat (marbling), surface moisture, tyrosine crystal coverage, odor intensity, hardness,

solubility, salinity, sweetness, acidity, bitterness, vegetable, smoky, spicy, rancidity, mold, soil, animal, rotten egg. Each attribute was evaluated on a 10 cm non-structured linear scale (0 = complete absent, 10 = maximum perception) on a whole dry-cured slice including 1 cm subcutaneous adipose tissue. Results showed that dry-cured ham from four different processing methods could be distinguished effectively by the PCA results of descriptive sensory analysis. Dalmatinski, Drniški, Istarski and Krčki pršut differ in appearance (fat color, uniformity of color) ($P \leq 0.05$), taste (salty, sweet, spicy and smoked) ($P \leq 0.01$) and texture (solubility) ($P \leq 0.05$). The color of muscle tissue is slightly higher in Istarski pršut, marbling is moderately intense and did not show differences in the studied dry-cured hams. Dalmatinski and Drniški pršut are characterized by a smoke aroma, while Istarski and Krčki pršut are characterized by a spicy aroma. Drniški and Dalmatinski pršut are also characterized by fat color, uniformity of color and sweetness, while Istarski and Krčki pršut are also characterized by solubility. In conclusion, this study shows a remarkable influence of production method on the sensory profile of Croatian dry-cured hams.

Keywords: Sensory profile, Istarski pršut, Dalmatinski pršut, Krčki pršut, Drniški pršut, dry-cured ham

SESSION 5

SOCIO-ECONOMIC ASPECTS

MAIN LECTURE

Stakeholders' perception of the local breeds' sector in six European countries - a survey by the GEroNIMO project

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As part of the European multi-actor project GEroNIMO (Genome and Epigenome eNabled breedIng in MOnogastrics) a survey was conducted with actors and stakeholders (breeders, advisers…) of the local pig breeds' sector to collect their perceptions. The objective was to describe the farms, their levels of productivity, their economic viability and the breeding programs. It also aimed to identify stakeholders' concerns about the future of local breeds and the overall impact of the sanitary

crisis on them. A dedicated online survey was designed in seven languages including English, with emphasis on six European countries: Croatia, Italy, France, Portugal, Slovenia and Spain. In some cases, face-to-face interviews were also conducted. A total of 374 responses were collected, of which the most complete (n=337) were included in the analysis. Results show that local pig breeds are mainly reared on small farms, using outdoor rearing, locally available feed resources and natural mating. Preserving genetic diversity and maintaining breeds' standard characteristics are the main motivations stated by the surveyed stakeholders. For one third of the surveyed breeders, activities related to local breeds are economically unbalanced, with deteriorating profitability due to the sanitary crisis. Genetic evaluation is performed in very few local breeds, but stakeholders demonstrated their interest for selective breeding, especially for reproductive traits. They expect the public authorities to protect the use of breed names and introduce incentives to increase productivity. A similar survey was also conducted for local chicken breeds.

Keywords: local pig breeds, stakeholders, survey, management program, genetics

Acknowledgement

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SESSION 5

SOCIO-ECONOMIC ASPECTS

ORAL COMMUNICATIONS

Protection of biodiversity and breeding of the Black Pig of Parma and of the Cinta Senese PDO in the Tuscan-Emilian Apennines

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The Italian Parliament adopted the legislation (National Law 1st December 2015, n. 194) called "Provisions for the protection and enhancement of biodiversity of agricultural and food interest" which implements the national plan and guidelines for the conservation of plant, animal and microbial biodiversity of agricultural interest", and establishes the principles for the protection and enhancement of biodiversity thus protecting the genetic resources from the risk of extinction and genetic erosion. The aim of the Law is the support to rural territory to help limit the phenomena of depopulation and preserve the territory from genetic pollution and loss of genetic heritage. The theoretical approach on which this research is based resides in the welfare economics and in recent developments on the circular economy. The research presents the case of the *Comunità del Cibo di Crinale 2040* (Community of Food of Crinale 2040) association for social promotion (APS) as an experience in the application of the national regulation for the protection of biodiversity; the Community includes the Tuscan, Emilian and Ligurian ridge, including the municipalities of Valdimagra and Valdivara, the Taro, Parma and Enza valleys of the Parma Apennines bordering the Lunigiana. The production of the Black Pig of Parma and Cinta Senese DOP is situated in the described territory. The Black Pig of Parma, recognized as a breed by the Ministry of Agricultural Policies (Mipaaf) with Ministerial Decree 11781/2016 and consequently included in the registry, has a good aptitude for grazing and high fertility. The Cinta Senese

obtained a recognition as a breed with Ministerial Decree 20871/2001 and the establishment of the relative Registry at the Mipaaf. Cinta Senese is part of the protected designation of origin (PDO) value-chain with the EU Reg. n. 217 of 13.03.12 and publication in Official Journal of the European Union of 15.03.12. The Cinta Senese PDO requests that animals are born, raised and slaughtered in Tuscany, at an altitude of up to 1,200 meters above sea level, and reared in free-range or indoor/outdoor combined systems. This research analyzes the data of the companies in the sector, considering the number of companies, reared pigs and their location within the Community. The study describes an example of the application of rules for the protection and promotion of biodiversity and the related branding with territorial development objectives that can be replicated in other territories of the Mediterranean basin.

Keywords: Law 194/2015, Circular Economy, Black Pig of Parma, Cinta Senese DOP, *Comunità del Cibo di Crinale 2040*

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We wish to tank *Comunità del Cibo di Crinale 2040* and IFCQ Certificazioni srl for the statistical data made available.

Consumer's opinions about societal concerns when purchasing fresh pork from the Black Slavonian Pig

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The main objective was to obtain an overview of consumers' opinions and beliefs on some aspects related to societal concerns (sustainability, biodiversity, rural development, and animal welfare) when purchasing fresh pork of Black Slavonian breed. Croatian consumers were recruited in June 2021 by an online survey (Qualtrics Inc.). The following criteria were used to select consumers: gender, age and geographical region of the Croatian population. The number of respondents was previously determined with company Qualtrics Inc. to represent the Republic of Croatia. The recruited Croatian consumers (n = 410) were asked to express their opinion on the statements referring to the Black Slavonian Pig regarding pork purchase, local production, preservation of biodiversity, and animal welfare. Data were analysed using descriptive statistics (frequency analysis, arithmetic mean, mode, median and standard deviation) and parametric tests (independent samples t- test, one-way ANOVA). The responses were measured using a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). Based on the respondents' answers, the average score to all statements was 3.80 (somewhat agree). Respondents most strongly supported the statement that breeding indigenous pig breeds promotes sustainability and supports local producers (mean = 4.26) and that locally produced food helps preserve traditional breeds and production methods (mean = 4.22). The statement that it should be clearly indicated on the product which breed the pork comes from was supported by 190 (46.3 %)

respondents, while 191 (46.6 %) of them agreed with the statement that outdoor rearing Black Slavonian Pig is environmentally more friendly. The results of a t-test and ANOVA show that there is a significant difference between socio-demographic characteristics of respondents in purchasing, and consumption habits, as well as their knowledge about the breed and attitudes towards investigated societal concerns. From the results of the study it is evident that consumer preferences and purchasing decisions are not primarily based on taste and health concerns, but they are influenced by many other factors such as the animal welfare, environmental, cultural and ethnic factors. Nowadays, consumers do not buy food only for its primary function, but their purchase behaviour is influenced by extrinsic factors like naturalness, authenticity, origin and tradition. By knowing the consumer opinions and beliefs, the farmers will be able to differentiate their products and production systems in order to increase their competitiveness. These results are important for producers, retailers, and policy makers and they can use these findings to design and develop successful tools for promotion of fresh meat and meat products from the Black Slavonian Pig and position themselves in different consumer segments by emphasizing environmental, ethical and sustainability aspects of their products.

Keywords: Consumer opinions, fresh meat, Black Slavonian Pig, societal concerns, Croatia

Protected Designation of Origin (PDO) label for pork of local breeds - the case of Turopolje pig

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The Turopolje pig (TP) is an endangered Croatian breed traditionally raised in open ecosystems of oak forests and wetlands of the Turopolje region. Recent research with Croatian consumers and stakeholders has shown that the TP breed needs to be valorised with value-added products to enable a more sustainable breeding. Therefore, a new conservation strategy was proposed based on the traditional production system and the niche of labelled products. Within this strategy, the Noble Municipality of Turopolje - Section of Turopolje Pig Breeders - has recently applied for the EU Protected Designation of Origin (PDO) label for fresh meat from TP (*PDO "Meso turopoljske svinje"*). The main points on which the PDO application is based are characteristics of the TP meat essentially linked to the terroir, including natural and human resources. In the case of TP, these are the relief-geographical, climatic and biotic factors of the local lowland forest ecosystems, which favoured the early development of pig breeding in the Turopolje area, whose inhabitants have been well-known pig farmers for centuries. Moreover, TP, as one of the oldest European pig breeds, has developed without major external influences over a long period of time, during which the continuous interaction between genotype and environment has adapted the breed to the use of natural resources. The production system with grazing and use of naturally available feed sources, the innate breed characteristics such as robustness, slow growth and the ability to produce fat, as well as the absence of intensive selection and crossbreeding with other breeds affect the development of body tissues and typical characteristics of the TP meat. For instance, higher muscle activity and slaughter age result in greater accumulation of muscle pigment, which is reflected in a darker

and more reddish meat colour. Meat and fat colour is also more stable due to more diverse food choices and intake of natural antioxidants and other ingredients that help stabilise tissues. The smaller diameter of the muscle fibres provides a finer texture to the meat, which is compact and has better water holding capacity than conventional pork. In addition, the meat of TP naturally has a higher degree of lipid deposition, especially in the subcutaneous fat and between the muscle tissues. As a result, the cooked meat has an elastic-juicy texture, a full flavour and a specific aroma of dissolved meat fats when eaten. Due to traditional free-range farming and distinctive meat and fat quality, the meat from TP is considered better and more appreciated than conventional pork by most of today's consumers, especially local ones.

Keywords: Turopolje pig, pork meat, food labels, geographical indications, PDO

POSTER SESSION

Antioxidants addition in the diet of pregnant sows modifies the expression of genes related to ovarian functionality in their progeny

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Age at puberty is an important productive criterion in the selection of future breeding sows, since it affects the productive life. It has been observed that the age at puberty is directly related to health and vitality aspects during the first moments of life, which could be associated to the oxidative status of the pregnant sow. Dietary supplementation of pregnant sows with antioxidant agents (vitamin E and hydroxytyrosol) improves the function of the placenta (antioxidant capacity and vascularization) and increases the weight and viability of piglets at birth. However, other effects on the progeny have not been studied. Therefore, the aim of this study was to evaluate expression of genes related to ovarian function in gilts born from sows supplemented with vitamin E and/or hydroxytyrosol during pregnancy and lactation. Four equal experimental groups were organized, based on the supplementation from day 85th of gestation until weaning of 50 pregnant Iberian sows: control (C) (30 mg VitE/kg feed); supplemented with vitamin E (VitE) (100 mg VitE/kg); supplemented with hydroxytyrosol (HT) (30 mg VitE+1.5 mg HT/kg) and supplemented with both antioxidants

(VitE+HT) (100 mg/kg VitE+1.5 mg/kg HT). When the piglets were 110±5 days old, 10 female piglets born from each of the four experimental groups (n = 40) were surgically castrated, ovarian tissue was collected and preserved for gene expression analysis by RT-qPCR. Genes involved in lipid metabolism pathways, steroid hormone metabolism and synthesis, as well as follicle-stimulating hormone receptors (*ACLY, SCD, ME1, RUNX1, IGF1, STAR, CYP11A1, LHCGR, FSHR, FST, HSD17B7*), associated with ovarian function, were selected for gene expression analysis. Statistical analyses of expression data were performed using the SAS 9.4 statistical package, where diet was included as fixed effect. We observed higher expression of *RUNX1, HSD17B7, FSHR* and *IGF1* genes in the VitE group compared with the remaining ones, but the differences were significant only when compared with the HT group (p=0.01, p=0.006, p=0.049, p=0.036 respectively). *LHCGR* (p=0.107) and *CYP11A1* (p=0.091) genes showed the same trend in the cited comparison. On the other hand, genes involved in lipid metabolism (*ACLY, SCD* and *ME1*) and synthesis of pregnenolone from cholesterol (*CYP11A1* and *STAR*), showed higher expression in the C group than the other ones, being significant when compared to HT (p=0.01, p=0.01, p=0.01, p=0.01 and p=0.03 respectively). HT group showed the lowest level of ovarian expression for all studied genes. Results showed consistent gene expression patterns in functionally related genes involved in lipid synthesis and ovarian hormone signaling. In turn, they suggest a greater potential for follicular development and maturation, steroidogenesis and growth of germ cells in the VitE group.

Keywords: Iberian sow, antioxidant, vitamin E, hydroxytyrosol, puberty, ovarian function

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Outdoor finishing of intact male pigs of local breed on a high fibre diet: effects on growth, carcass, and some meat quality traits

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Alentejano (AL) pig is a Portuguese local breed known for the quality of its meat and meat products obtained from heavy pigs, traditionally surgically castrated without pain relief, but due to current issues related to this procedure, other solutions must be researched to prevent/reduce boar taint. One approach is diet manipulation. In this trial, 30 male AL pigs raised outdoor with *ad libitum* water and feed were used to test the effects of a new high soluble dietary fibre feed on animal growth, carcass traits, and meat quality. Pigs were individually fed commercial diets from ~40 until 130 kg body weight (BW). From 130 kg until slaughter (160 kg BW), surgically castrated (group C) and intact pigs (group I) were fed commercial diets, while another group of intact pigs was fed an isoproteic and isoenergetic experimental diet (CP ≈ 14 %, Digestible energy ≈ 13.1 MJ/kg DM), containing agro-industrial by-products (group IE). Overall, average daily gain was higher in IE and I pigs than in C pigs until ~130 kg BW and higher in IE than I and C pigs between 130 and 160 kg BW (732 in IE, and 595 and 591 g/d in I and C pigs respectively, $P<0.05$).

Both intact groups (IE and I pigs) also presented leaner carcasses, with higher lean to fat cuts ratio (1.98 and 1.96 in IE and I, and 1.63 in C pigs, P<0.001) and lower last rib backfat thickness (48.5 and 45.3 in IE and I, and 63.7 mm in C pigs, P<0.001). *Longissimus lumborum* (LL) pH_u was different between I and C groups (5.61 in IE, 5.58 in I, and 5.68 in C pigs, P<0.05). There were also differences between both intact and C groups in LL moisture content (73.0 and 73.5 in IE and I, and 71.4 g/100 g in C pigs, P<0.001) and intramuscular fat (IMF) (2.96 and 2.72 in IE and I, and 4.12 g/100 g in C pigs, P<0.01). Finally, LL total protein, ashes, myoglobin content and CIE *a** (redness) were not different among groups. *Psoas major* (PM) pH_u was similar between experimental groups and moisture content different between both intact and C groups (74.5 and 74.4 in IE and I, and 73.5 g/100 g in C pigs, P<0.001). PM IMF was also different between both intact and C groups (1.79 and 1.94 in IE and I, and 2.40 g/100 g in C pigs, P<0.001). PM total protein and ashes were not different among groups. Finally, PM myoglobin content was lower in both intact groups (2.66 and 2.75 in IE and I, and 3.19 mg/g in C pigs, P<0.01). Overall, these data show that the experimental diet had no negative effect on the growth of intact AL pigs when compared to the one obtained in intact AL pigs consuming commercial diets. Intact AL groups produced leaner carcasses and meat than castrated ones. Further studies will test the effect of the experimental high fibre feed on pork boar taint and fatty acid profile of meat and fat of intact AL heavy pigs raised outdoors.

Keywords: Alentejano pig, intact pigs, growth, carcass, meat quality

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Effect of fish oil supplementation of the Iberian sow on piglet performance during lactation

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Autochthonous pig breeds are generally less productive than their selected counterparts. The main reasons for this problem in Iberian pig production are low prolificacy and low growth rate, especially in traditional extensive farms. These factors result in a low number of weaned piglets, with low and highly heterogeneous weaning weights. Therefore, it is necessary to develop nutritional or management strategies in order to increase piglet growth and survival. This study aimed to evaluate the effects of fish oil supplementation in Iberian sows performance during the lactation phase. Iberian sows were either offered a commercial diet, referred to as control diet (Ctrl; n=17) or a diet supplemented with an n-3 long chain fatty acid (n-3 LCFA; n=19) from mating up to weaning. Sows were weighed before mating, when entering the farrowing unit, and finally at weaning. The number of Iberian piglets were registered at birth (born alive, dead and mummies), as well as the alive ones at 21 days and at weaning, which took place at 30 days of life. SAS software was used for statistical analysis. MIXED procedure was used to perform the analysis of variance of the different continuous variables and the GLIMMIX procedure was used for discrete variables. Piglets were weighted at birth, at 21 days and at weaning. Preliminary results showed no differences in Iberian sows´ weights or in the number of piglets at birth and throughout lactation. Piglet weight at birth was higher in Ctrl sow litters than in n-3 LCFA sow litters (p= 0.001).

However, piglets from n-3 LCFA sows had greater weight at weaning ($p = 0.028$) and average daily gain ($p = 0.007$) from birth to weaning. In conclusion, fish oil supplementation of Iberian sows improved the growth of Iberian piglets during lactation which might favour a greater piglet growth and survival rate during the post-weaning phase.

Keywords: Autochthonous pigs, n-3 LCFA, fatty acids, piglets, weaning

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Animal and environmental factors affecting reproductive traits in Nero di Parma sows

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Nero di Parma is an Italian swine breed recognised in 2016, after being recovered from the original type "Nera Parmigiana" which was extinct in 1970s due to crossbreeding with highly productive breeds. The recovery project originated from the growing interest for outdoor farming and the awareness that local breeds are a key genetic resource that should not be lost. Local breeds share relevant phenotypic characteristics such as resilience, adaptation to rough environments and high meat quality, which make them a valuable genetic resource to be preserved. The aim of the present study was to detect how reproductive traits are influenced by animal and environmental factors in Nero di Parma swine breed. The dataset was built following all the reproductive events from 2013 to 2021 and consisted of 811 litters from 300 different sows. The litter size, the mortality rate and the number of piglets weaned were evaluated in function of i) season (spring, summer, autumn and winter), ii) inbreeding, divided in quartile classes, iii) year (from 2015 until 2021), iv) herd (seven in total) and v) parity order (divided in primiparous and multiparous). The statistical analysis was performed using the ASReml software (version 4.1) with a univariate animal model. The mean number of piglets weaned per litter was 6.97 while the mortality rate was 0.82 piglets/litter. Average litter size was 7.7 piglets until 2019, reaching 8.4 piglets/litter ($P<0.01$) in 2021, highlighting the increase of reproductive efficiency in the breed. Season had an influence on reproductive parameters, since in summer there was higher mortality rate ($P<0.05$), while in spring larger litters were observed ($P<0.001$). However, the number of newborn piglets mainly depended on the herd management

(P<0.001). These preliminary results suggest that reproductive parameters of Nero di Parma sows were affected by season, herd, year, and parity order but not by inbreeding. Nero di Parma swine breed is well adapted to semi-extensive conditions and herd management seems to have major influence on reproductive parameters. Further studies considering larger numbers of animals will provide additional information to the breeders in order to better manage the reproductive efficiency of sows. The provided data will contribute to understand the effect of environmental factors in a small swine population as the Nero di Parma breed, and to promote and preserve the genetic reserve of local swine breeds in Italy.

Keywords: Local breeds, reproductive traits, environmental effect, genetic diversity

Effect of long term heat stress on nutrient digestibility of Iberian pigs

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Heat stress in pigs is characterized by decreasing feed intake and body weight gain, as well as damaging small intestine and altering gut microbiota. The aim of the present study was to evaluate the effect of constant heat stress on nutrient digestibility in Iberian pigs. Twenty-four Iberian barrows (44.0 kg body weight) were used to estimate how constant heat stress (30ºC) may affect nutrient digestibility. At arrival, pigs were individually housed in a room at 22ºC and fed *ad libitum* an experimental diet based on barley-corn-soybean meal covering all nutrient requirements. Pigs were assigned to three treatments for 14-days: 1.-thermoneutral (22 °C) and fed *ad libitum* (TN); 2.-heat stress (30 °C) and fed *ad libitum* the control diet (HS); 3.-thermoneutral-pair-fed on the basis of intake of HS group to eliminate confounding effects of dissimilar feed intake (TN-PF). After one week, pigs were moved to individual metabolic crates for 5 days (1 day of adaptation and 4 days of total urine and feces collection). The collected feces and urine were weighed and stored at -20ºC. At the end of the digestibility measurements, composite samples of feces and urine were obtained separately for each pig for the entire experimental period. Fecal samples were freeze-dried and grounded for further chemical analysis. Apparent total tract digestibility of dry matter, organic matter, energy, and nitrogen were measured. Apparent digestibility coefficients for nutrients and energy were calculated using standard procedures. Treatment effect was evaluated using the GLM procedure of SAS. Significant differences between treatment means were assessed by Tukey t-tests. Results were

considered significant at $P < 0.05$. Pigs subjected to heat stress had increased ($P = 0.003$) digestibility of dry matter (0.845) compared to TN (0.823) and TN-PF (0.825). Organic matter digestibility significantly ($P = 0.002$) also increased in HS pigs (0.864) compared to TN (0.843) and TN-PF (0.846). Digestibility of nitrogen increased ($P = 0.004$) under heat stress conditions (0.754) compared to their thermoneutral counterparts (0.718 and 0.723 for TN and TN-PF, respectively). Digestibility of energy tended to increase ($P = 0.061$) after heat stress (0.844) in comparison to TN-PF (0.834), with TN pigs having intermediate values (0.813). Preliminary results indicate a positive effect of long term heat stress on nutrient and energy digestibility in Iberian pigs. Similar information in contemporary pig breeds is contradictory.

Keywords: Fecal digestibility, heat stress, Iberian pig

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The Apulo-Calabrese pig breed: a valuable scientific, economic, and social heritage

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The Apulo-Calabrese breed is included in the list of endangered breeds of the United Nations Food and Agriculture Organization and registered in the herd book held by the National Association of Pig Breeders (National Association of Pig Breeders, ANAS). In 2017, the breeding population numbered 540 sows and 63 boars reared in 63 farms, 31 of which were located in Calabria. The Apulo-Calabrese is a medium-sized, black-skinned pig with small socks on the forelimbs and large socks on the hind limbs. It is well adaptable to be kept outdoors or indoors in a conventional system. The meat of the Apulo-Calabrese is transformed into four salamis with Protected Designation of Origin (PDO), typical of the Calabrian region (*Salsiccia*, *Soppressata*, *Capocollo* and *Pancetta*). Only few scientific data is available onthe Apulo-Calabrese pigs as, until now, it has been of interest only locally. The aim of this study is to review the main productive and reproductive data available in the scientific literature for the Apulo-Calabrese breed.

Keywords: Apulo-Calabrese, meat quality, PDO, biodiversity.

"Green deal" as a framework for the development of sustainable pig production in Croatia

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The guidelines for the future development of pig production in Croatia should be based on the strategic policies of the "Green Deal". Due to its specific resources, which primarily refer to traditional heritage, land structure and geographical and climatic determinants, Croatia has a unique opportunity to significantly implement the "Green Deal" in its development and strategic guidelines, when talking about the pig sector. Defining alternative production systems, such as deep litter and free range systems, and integrating them into the "Farm to fork" system could profile Croatia and its pig farming as a production area of high-quality pork products, in a way that meets the criteria of pig welfare and health, conservation of biodiversity and ecosystems, survival and retention of people in rural areas, and development of a circular economy with increased productivity. Keeping pigs in deep litter has a number of positive effects. Reducing production costs by constructing cheaper and more functional farms, with positive impact of straw on animal welfare and health, production of significant quantities of quality manure as a key factor in restoring soil fertility, and production traditional high value-added meat products, all that can significantly contribute to better profitability for producers, especially in times of global crisis, as we are witnessing today. Traditional pig feeding systems, which include maximum utilization of the potential of local resources in the production

of feed and by-products, can also significantly contribute to better management in crisis situations. Therefore, the focus on production systems in which the emphasis is on product quality rather than intensity and quantity is key for achieving such goal. This would also enable Croatia to position itself on the global market as a place of production of quality and healthy pork and pork products in conditions that are economically and environmentally sustainable and acceptable from the point of view of welfare and health of pigs. Keeping pigs in deep litter and keeping them outdoors are a very suitable production system for use on family farms in Croatia, which should be responsible for the development of pig farming.

Keywords: Green deal, alternative production systems, deep litter, animal welfare, sustainability

Physiological effects of androgen deprivation in the late sexual development phase (model of adult boars)

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Androgen deprivation in male prepubertal pigs by immunocastration has been systematically studied in recent years due to increased awareness of the negative effects of surgical castration without pain relief on animal welfare. In contrast, information on the physiological effects of immunocastration in sexually mature animals remains limited. Therefore, a study was conducted with old immunocastrated (IC) boars (n=19), young male IC pigs (positive control; n=6), and young male pigs (EM; negative control; n=6) to compare the effects of immunocastration in sexually mature boars with those observed in male prepubertal pigs. Animals included in the study were immunocastrated with Improvac® with the second dose administered 4 weeks prior to slaughter. Parameters related to sexual development were analysed and compared between groups to evaluate the extent of immunocastration in old IC boars; the following parameters were evaluated: testosterone concentration at slaughter (ng/ml plasma), luteinizing hormone concentration at slaughter (ng/ml plasma), colour of testicular parenchyma, testicular index calculated as the testis weight (weight of right and left testis including epididymis) divided by warm carcass weight, response to immunocastration (androstenone concentration in backfat (µg/g liquid fat), gonadotropin-releasing hormone (GnRH) antibody binding at slaughter (%) and histomorphometric analysis of testicular tissue (nucleus- to- cytoplasm ratio in Leydig cells calculated as cytoplasmic area divided by nucleus area, volume density of Leydig cells in testicular parenchyma). The increased GnRH antibody titer ($p < 0.001$), decreased serum luteinizing

hormone concentration (p=0.002), and vascularization of testicular tissue (lighter and less red testicular parenchyma; p≤0.001) observed in IC old boars indicate that all old IC boars responded to vaccination, but with different responses. A significant reduction in testicular index ($p < 0.001$), Leydig cell volume density ($p < 0.001$), nucleus to cytoplasm ratio in Leydig cells ($p < 0.001$), and testosterone concentration ($p < 0.001$) was observed 4 weeks after GnRH suppression in 10 of 19 (53%) old IC boars. However, in 9 animals this period was not sufficient to elicit a complete immunocastration response; these animals may require an additional booster dose or a longer period between revaccination and slaughter, which should be tested in future studies.

Keywords: Male pig, immunocastration, testicular histomorphology, gonadal hormones

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Effect of arginine supplementation in the Iberian pig on performance during the lactation and post-weaning periods

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The new EU regulations restrict the use of antibiotics and zinc oxide as piglet feed additives, which have been used to prevent the post-weaning diarrhea favored by sudden nutritional changes and the regrouping stress. This problem is especially important for the Iberian pig, due to its low prolificacy and slow piglet growth. Therefore, it is necessary and urgent to find alternative strategies to these antimicrobial additives. In several studies, arginine supplementation has been beneficial for certain litter parameters at birth and also for pre-weaning intestinal maturation. This study aimed to evaluate the effects of supplementation of sows, piglets or both with arginine during the gestation and lactation phases in the Iberian pig. Iberian sows were either supplemented with arginine (Treated=Trt; n=23; 8 g/kg feedstuff; 16 g/head/day) from day 50 of pregnancy up to weaning, or were used as Controls (Ctrl; n=23). Sow body weight (BW) and ultrasonographic subcutaneous fat thickness at the inter-scapular and gluteal areas were measured before mating, at farrowing and at weaning. Piglets were not treated with antibiotics or ZnO. In Ctrl or Trt sow litters, pre-weaning (*creep feeding*) and post-weaning feed were supplemented with arginine (8 g/kg feedstuff) from day (d) 15 to weaning (d28) and up to post-weaning day (pwd) 28, with or without arginine (*trt* or *ctrl*, respectively). Therefore, there were 4 types of piglets: Trt-*trt* (n=5), Ctrl-*trt* (n=15), Trt-*ctrl* (n=10) and Ctrl-

ctrl (n=10). Piglet BW was recorded at birth, d21, d28, pwd14 and pwd28. Individual dry matter of feces was determined on pwd7, pwd14, pwd21 and pwd28. Additional piglets (12 Trt-*trt* and 14 Ctrl-*ctrl*) were slaughtered at weaning to collect ileal tissue for villi measurement. Blood was collected on d28 (dam and piglets) and pwd28 (piglets) for metabolic analyses. Preliminary results showed that litter size was smaller (in number of total born and born alive piglets) for Treated sows. This deleterious effect could be due to an excessively early arginine supplementation during pregnancy, although arginine was given earlier in the previous studies reporting litter size reduction in other breeds. There was no effect of treatment on BW or subcutaneous fat thickness of sows. As for piglet development, no differences in BW were found between *ctrl* and *trt* piglets. There was a trend for a greater dry matter percentage of *trt* piglets on pwd7, when diarrhea was more prevalent in all groups. The effect of arginine was clearer when analyzing the 4 piglet groups separately, as the fecal dry matter percentage was greater in Ctrl-*trt vs* Ctrl-*ctrl* piglets on pwd7 and also greater in Trt-*trt* vs Ctrl-*ctrl* on pwd14. In conclusion, preliminary results suggest that arginine supplementation to sows was deleterious during pregnancy and was not very useful during lactation. In contrast, arginine supplementation to piglets increased fecal consistency on key days for diarrhea outbreaks after weaning, regardless of dam treatment for the most critical period. Therefore, pre- and post-weaning supplementation of Iberian piglets with arginine may be a practical way to avoid antimicrobial treatments to prevent post-weaning diarrhea.

Keywords: Pregnancy, litter, piglets, diarrhea, antibiotic restriction

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Effect of genetic type on tissue composition and meat quality traits in Nero di Parma pig breed and its cross with Casertana

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Nero di Parma is an Italian local pig breed, still reared in Parma's area and well appreciated by consumers whose demand for local and traditional pork products has established a niche market for this breed. However, at slaughter animals are characterized by heterogeneous weight and carcass conformation. Also, meat quality traits such as intramuscular fat are often inadequate for transformation. Hence, the aim of the present study was to assess the effect of crossing Nero di Parma with Casertana, another Italian local pig breed with good carcass conformation and excellent meat quality traits, to enhance carcass conformation and composition, as well as to improve meat quality. Seven Nero di Parma (NP) pure breed pigs and seven crossbreed Casertana x Nero di Parma (CA x NP) pigs were reared outdoor and fed with commercial concentrate mixture in the amount of 3% of live weight. Once animals reached 160±10 kg of live weight were slaughtered and carcass weight, backfat thickness and main cuts percentages were assessed on the left carcass side. From each left carcass side, a portion of loin from the 2nd to 5th lumbar vertebra was taken and dissected into meat, fat, and bone. Furthermore, a sample of *longissimus dorsi* was stored to perform analysis on meat quality. The parameters assessed were pH_{24}, instrumental texture, CIE colour, chemical composition, fatty acid profile, and, on ham, weight loss after salting. Data were analysed by SAS software with analysis of variance (ANOVA). Results on carcass weight and backfat thickness were the same for NP and CA x NP animals. Similarly, sample joint's tissue composition was not affected by genotype except for

Psoas major weight, which resulted greater in NP than in CA x NP animals (p<0.030). Concerning meat quality traits, pH, and raw and cooked WB shear force were not affected by genotype. Texture profile analysis only identified differences between NP and CA x NP meat for cooked meat chewiness, significantly higher for CA x NP group (p<0.005). Meat colour was comparable between the two groups, while significantly higher L* was assessed for fat of NP animals (p<0.040). In line with the previous results, also chemical composition of meat (moisture, protein, fat, ash contents) resulted similar, as well as the amount of cathepsin B and iodine number were the same between the two groups. Fatty acids profile of fat and meat was also investigated. NP pure breed pigs showed lower content of oleic acid (p<0.040) and, thus, lower MUFA percentage in fat (p<0.020). In meat, NP group resulted in higher stearic acid (13.9 % *vs.* 11.9 % in crossbreed pigs) (p<0.005) which entailed higher SFA % (40.7 *vs.* 37.6 in CA x NP group) (p<0.020) and lower MUFA % (44.5 *vs.* 46.4 in CA x NP group) (p<0.050). Lastly, genotype mainly affected ham weight loss after salting, which resulted significantly higher in CA x NP hams, both after first and second salting (p<0.010).

Keywords: Pork, intramuscular fat, local breed, fatty acids, ham

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Carcass traits of Mangalitsa pigs – effect of immunocastration

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This research was aimed at evaluating carcass differences between surgical and immunocastrated fatteners of Mangalitsa breed. The study comprised 23 male pigs kept under the identical management conditions and divided in the two groups: surgically castrated (SC, n=11) and immunocastrated (IC, n=12). The animals were kept in a semi-outdoor system facility with open (110 m^2) and covered (40 m^2) part. Pigs under investigation were fed complete feed mixtures (15% and 13% of crude protein and 13.5 MJ ME/kg). Carcass traits measured on the slaughter line included: warm and cold carcass weight (kg), carcass length (cm), share of the main parts in half-carcasses (ham, loins, shoulder, rib-abdominal part; %), fat and *longissimus dorsi* muscle thickness (mm). Thickness of subcutaneous fat measured at three points: where *m. gluteus medius* gets deepest into the fat tissue; at the level of the first rib – between the last neck and the first thoracic vertebrae; at the level of the last rib – between the last thoracic and the first lumbar vertebrae. Muscle thickness measured from the end of the spinal column to the cranial part of the *m. gluteus medius*. At slaughter, SC and IC group of pigs had live weight of 116 and 121 kg, respectively ($p>0.05$). Warm and cold carcass weight in SC, compared to IC group, was higher by 2.2, i.e. 2.4 kg, respectively ($p<0.01$). The length of one carcass half measured from the anterior edge of pudendal bone up to an atlas cranial edge was similar (100 and 99 cm in IC, i.e. in SC group, respectively; $p>0.05$). The fatteners in the IC group had a higher share of carcass ham (11.4 vs. 9.9%, $p<0.001$). Loins share in halves was greater in SC group (+1.1%, $p<0.05$).

Thickness of subcutaneous fat at three points was higher ($p<0.01$) in SC group by 12.2, 11.7 and 11.6 mm. *Longissimus dorsi* muscle thickness was 61.9 and 57.5 mm in the SC and IC group ($p>0.05$), respectively. The immunocastrated group had better carcass traits, considering that they had a higher share of ham in carcass and lower fat thickness.

Keywords: Pig, immunocastration, ham, fat thickness, *longissimus dorsi*

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Influence of on-farm resting time on some meat quality parameters in sows

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The aim of this study was to determine the influence of the on-farm resting period (RT) prior to slaughter in an on-farm outdoor facility on the meat quality parameters of culled sows. The study was conducted on 24 Large White x Landrace sows, divided into three groups according to the duration of RT: control group (n=10, CG) with sows destined for slaughter without on-farm RT; first experimental group (n=7, E1) with sows destined for slaughter after 14 days RT; and second experimental group (n=7, E2) destined for slaughter after 28 days RT. The sows entered the experiment after two to four farrowing at approximately 2 years of age and 250 kg live weight (LW). During the trial, sows from E1 and E2 groups were housed in the same conditions and fed the same diet with *ad libitum* access to feed and water. After 0-, 7- or 14-days RT, animals were transported to a nearby abattoir (less than 1 hour) where they were slaughtered without lairage. To enhance food safety and meat quality animals were fasted for 18h prior slaughter. After exsanguination following stunning with CO_2, the following meat quality characteristics were determined: pH and electrical conductivity 45 minutes and 24 hours after slaughter in the *longissimus thoracis et lumborum* (LTL) and in *the semimembranosus* muscle (SM), together with colour (CIE L*a*b*), drip loss and instrumental tenderness of the LTL muscle. The RT influenced nearly all meat quality traits studied, with the exception of electrical conductivity measured 45 minutes *post mortem* and

instrumental tenderness. LTL and SM from CG sows exhibited significantly higher ($p<0.05$) pH values measured both 45 minutes and 24 hours after slaughter than meat from groups E1 and E2. E1 and E2 did not differ from each other in these traits. Although no significant differences were found between investigated groups for CIE L*, the LTL muscle of the CG sows tended to be darker ($p=0.06$) than the LTL of the E2 group. The LTL muscle of the E1 group exhibited the highest ($p<0.05$) CIE a* and CIE b*, while the lowest values for both colour coordinates were observed in the CG group. The meat from the CG group had also the lowest drip loss ($p < 0.05$), while E1 and E2 did not differ in this trait. The results of this study indicate that even a 14-day rest/recovery period of the sows on the farm can significantly improve their meat quality traits.

Keywords: Sows, meat quality, recovery time, slaughter

Intramuscular fat contents in Iberian pig: Relationship with productive performance and in vivo estimation by ultrasound technique

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Intramuscular fat is one of the most important traits for the quality of the meat and dry-cured products of the Iberian pig. It would be useful to know the intramuscular fat content *in vivo* prior to slaughter to decide if the pig is ready to provide high quality meat. It could also be of interest to know the development of muscle during the last fattening phase of the pig, and thus be able to make decisions about changes in the production phase aimed at increasing the intramuscular fat content (IMF). The objective of this work was to study the application of ultrasound *in vivo* to determine the amount of intramuscular fat in the *longissimus dors*i of intensively fattened Iberian pigs during the last fattening period. To this aim, eighteen Iberian × Duroc (50%) pigs were selected at the age of 260 days and with an average body weight of 114.2 ± 4.83 kg. For 53 days they were kept in group with *ad libitum* feed intake (3260 ME Kcal/kg, 9.85 % crude protein and 0.54% Lys, as fed basic). Feed consumption was recorded throughout the fattening period. The pigs were fed until the slaughter weight of 156.7 ± 8.19 kg was reached. Three days before slaughtering, pigs were restrained in a crate and an ultrasound image was taken using an ultrasonic device (EXAGO ultrasound scanner) equipped with a linear probe (13 cm long and 3.5 MHz) at 7 cm from the dorsal midline. The images covered the area between the 10th and 13th rib and were analysed to calculate the IMF using the tool BioSoft Toolbox® II for Swine (Biotronics Inc.) with a square Region of Interest (ROI) measurement of 66 × 66 pixels. After slaughtering, hot carcass and loin

were weighed and the backfat thickness at the level of the last and 10th rib was recorded. The IMF (in *longissimus dors*i sections from ribs 10th to 13th) was chemically analysed by Soxhlet method. The results were described using the mean ± standard deviation of the mean. Pearson correlation analyses (SPSS v 21) were performed between the IMF obtained by chemical methods and the other productive measures, as well as with the IMF obtained by ultrasound equipment. Statistical significance was established for $p<0.05$. The results obtained revealed that the pigs had and average daily gain (ADG) of 801± 106.5 g/day, a feed intake of 4.1 kg/day and a feed conversion ratio of 5.2 ± 0.67. The loins had an average individual weight of 2.7 kg with a carcass yield of 4.2 ± 0.3 %. The IMF analysed by chemical extraction was 3.95 ± 1.04 % (range 2.6 to 6.0). The IMF determined could only be explained by the weight of the animals at slaughter; so, when the slaughter weight increases, the IMF also increases ($r=0.59$, $p<0.02$). There was no significant correlation between IMF content measured ultrasonically at the end of the fattening period and IMF measured by chemical extraction. Therefore, it could be concluded that it would be desirable slaughtering Iberian × Duroc (50%) pigs at higher slaughter weights in order to achieve higher intramuscular fat content. The use of ultrasound technology to determine the IMF content needs to be more accurate in order to be used in more practical way.

Keywords: Intramuscular fat, Iberian pig, ultrasound

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A meta-analysis of fat composition in Cinta Senese and its crosses

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The aim of this study was to provide a further and broader comparison of Cinta Senese and Large White breeds and their crosses for fat composition. Data from three previous studies was used. These trials aimed at comparing the purebred and crossbred animals on different farming systems (indoor vs. outdoor) and stocking densities. A total of 198 records from 127 individuals were gathered and compiled into a single dataset, most of the individuals had two records since both inner and outer subcutaneous fat layers were analysed. Fat samples were extracted and analysed using gas-chromatography. The fatty acids identified were C12:0, C14:0, C16:0, C18:0, C20:0, SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA and were expressed as proportion to total fat. The model implemented for the analysis included the fixed effects of the trial (3 levels), the sex of the animal (2 levels: gilt and barrow), the layer (2 levels: inner or outer) and the breed (3 levels: purebred Cinta Senese, purebred Large White and their cross) as well as the random effect of the animal and the residual. Significance was declared for the *F*-test applied to the fixed effects at $P<0.01$. Results showed the effect of the breed being significant for C16:0, C18:0, C20:0, MUFA, n-3 PUFA and n-6 PUFA; the effect of the layer was significant for all groups; the effect of sex was significant for C20:0; the effect of the trial was significant for C12:0, C16:0, C18:0, C20:0, SFA, MUFA, PUFA, n-3 PUFA and n-6 PUFA. The breed Large White showed lower values than the other groups for C16:0 and C20:0, the crossbreds showed higher values than the purebreds for C18:0. Cinta Senese showed higher values for MUFA. For n-6 PUFA,

Large White showed higher values than the crossbreds and Cinta Senese, with the latter showing the lowest content.

Keywords: Cinta Senese, crossbreeding, meat quality, fat composition

Influence of smoking method and anatomical site on the content of polycyclic aromatic hydrocarbons in Turopolje ham

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To ensure a more sustainable breeding of the local Turopolje pig (TP), it is necessary to valorise its meat with high added-value products. Among the TP meat products that benefit most from processing, the dry-cured and smoked Turopolje ham stands out. To date, there are very few data on the quality and safety of Turopolje ham, including information on the content and distribution of potential contaminants such as polycyclic aromatic hydrocarbons (PAHs) from smoke. Hence, the aim of this study was to investigate the influence of smoking method (traditional or industrial) and anatomical site (*M. biceps femoris* - BF or *M. semimembranosus* - SM) on the content of PAHs in Turopolje ham. All hams (n=15) were produced the same way, differing only in smoking procedures; a traditional way above open fire (group TRAD, n=5) or in the smoking chamber (group IND: A - standard smoking, n=5, or B - 50% reduced smoking time, n=5). PAHs content (µg/kg) was determined and quantified by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Two-way analysis of variance was performed (PROC GLM of SAS) with fixed effects of smoking (S), muscle (M) and their interaction (SxM). Of the PAHs investigated, 14 compounds were detected: naphthalene (Nap), fluorene (Flu), acenaphthene (Acp), phenanthrene (Phen), anthracene (Ant), fluoranthene (Flt), pyrene (Py), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbFA), benzo[*k*]fluoranthene (BkFA), benzo[*a*]pyrene (BaP), dibenz[*a,h*]anthracene (DbahA) and benzo[*ghi*]perylene (BghiP). LOQ

ranged from 0.10 (BaP) to 3.30 μg/kg (Flu). The results showed that TRAD hams had higher levels of Ant, Chr, BbFA, BaP, and the sum of BaA, Chr, BbFA and BaP (PAH4) than hams from both A and B IND groups. TRAD hams had also higher content of Acp, Phen, Py, BaA, and total PAHs than IND B hams. Compared to BF, SM muscle had higher content of several PAHs i.e. Nap, Flu, Acp, Phen, Ant, Flt, Py, BaA, BkFa, BghiP, and also higher total PAH content. However, a significant SxM interaction was observed for Phen and Ant content, which were higher only in SM muscle of TRAD hams. In conclusion, this study shows a marked influence of smoking method and anatomical site on the PAHs content in Turopolje hams, mainly in the form of lower BaP and PAH4 content in all chamber smoked hams and higher light PAHs, some heavy PAHs, and total PAHs content in the more exposed SM muscle. Reduced smoking in IND hams did not affect BaP and PAH4 content, but reduced the content of some individual and total PAHs. Finally, it should be emphasised that two hams from the TRAD group exceeded the limit for PAH4 (30 μg/kg) set by EU legislation, indicating the need for more careful control of the traditional smoking process.

Keywords: Dry-cured ham, meat smoking, PAH, muscle, Turopolje pig

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Alternative packaging techniques for the valorization of Cinta Senese dry-cured ham

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The evolution of food packaging methods has great potential for reducing waste through chemical and microbial control of the product and/or storage environment. Cinta Senese pig products are characterized by a high fat content and a high proportion of unsaturated fatty acids, which are linked to genetic and husbandry factors and, consequently, involve a high risk of oxidation. So, the aim of the work was to test the storage stability of Cinta Senese dry-cured ham slices under three packaging categories (AP, Active Packaging; MAP, Modified Atmosphere; UV, Under Vacuum). A batch of 8 hams homogeneous for processing date, curing times and weight was used. The hams were entirely sliced. About 700 slices/ham (1 mm thick) were obtained and packaged under three conditions and, within each technique, between different storage times (zero, 20 and 40 days). During storage the slices were kept at a constant temperature of 2 °C. At the beginning of the trial, and in the different periods of storage time, the following analyses were carried out each time on 15 slices of each ham taken from the same section of these: moisture, colour, malonaldehyde content, proteolysis index, microbial growth and sensorial profile. To analyse data, GLM procedure was used with the following model: $Yijl = \mu + Pi + Tj + (PxT)ij + \varepsilon ijl$ where P is packaging fixed effect and T the storage time effect. The vacuum packaging recorded the best values for the colorimetric and oxidative parameters, while no statistical difference between the techniques occurred for the other parameters. The storage time, within each technique, significantly affected the colorimetric parameters and oxidative changes in the lipid component without, however, affecting the sensorial profile and the

shelf-life of the product. The study also showed that it is possible, regardless of the technique tested, to extend the packaging time to 40 days without any deterioration in the quality of the product.

Keywords: Packaging, dry-cured ham, shelf-life, quality, pig

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