

# A qualitative and participative approach to analyse different strategies of valorization of Noir de Bigorre pork chain

Justine Faure, Ludovic Brossard, Florence Garcia-Launay, Marta Gil, Zein Kallas, J Maria Gil

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X
INTERNATIONAL
SYMPOSIUM
OF
MEDITERRANEAN PIG

### **BOOK OF ABSTRACTS**

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X International Symposium of Mediterranean Pig

### **BOOK OF ABSTRACTS**

16<sup>th</sup> - 18<sup>th</sup> October 2019

Auditorium Camera di Commercio - Piazza Mentana, 1

Florence

Italy

X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

**ABSTRACT** 

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### **INDEX**

- FINAL PROGRAM	4
- SESSION I	15
Meat Quality and Food Technology	15
- MAIN LECTURE	16
- ORAL COMMUNICATIONS	18
- SESSION II	27
Animal Breeding and Genetics	27
- MAIN LECTURE	28
- ORAL COMMUNICATIONS	30
- SESSION III	42
Socio Economics	42
- MAIN LECTURE	43
- ORAL COMMUNICATIONS	45
- SESSION IV	51
Farming System – Animal Health	51
- MAIN LECTURE	52
- ORAL COMMUNICATIONS	54
- SESSION V	60
Animal Breeding and Genetics	60
- ORAL COMMUNICATIONS	60
- SESSION VI	65
Nutrition and Reproduction	65
- MAIN LECTURE	66
- ORAL COMMUNICATIONS	69
- POSTER SESSION	77



### **FINAL PROGRAMME**

Venue	Camera di Commercio di Firenze	
	Wednesday, 16/10/2019	
14:00-15:00	ARRIVAL - REGISTRATIONS	
15:00-16:00	Opening Session  Welcome Addresses  Organizing Committee  University of Florence  Tuscany Region  Animal Science and Production Association  Chamber of Commerce	
16:00-18:30	Meat Quality and Food Technology	
Chairmen	Bénédicte Lebret – David Tejerina Barrado	
16:00-16:30	MAIN LECTURE  Clemente López Bote  Distinctive characteristics of Iberian pig meat	101
16:30-16:45	Mónica Flores, Clarissa Salafia, José Javier López-Diez, Carmela Belloch Flavour generation in dry cured loins as affected by paprika addition and reduced nitrite/nitrate ingoing amounts	Q01
16:45-17:00	Danijel Karolyi, Urška Tomažin, Nives Marušić Radovčić, Zoran Luković, Dubravko Škorput, Krešimir Salajpal, Helga Medić, Martin Škrlep, Marjeta Čandek Potokar Effect of animal's diet, processing method and muscle type on quality traits of dry-cured hams produced from Turopolje pigs	Q02

17:00-17:15	Bénédicte Lebret, Carolina Pugliese, Marjeta Čandek-Potokar and TREASURE consortium  Quality of pork from European local pig breeds: analytical study on the average and variability of sensory, technological and nutritional traits within and between breeds - TREASURE project	Q03
17:15-17:30	Gizella Aboagye, Stefania Dall'Olio, Martina Zappaterra, Leonardo Nanni Costa Apulo-calabrese breed: Preslaughter-stress and meat characteristics	Q04
17:30-17:45	Miguel Angel Fernández-Barroso, Silvia Parrini, Patricia Palma- Granados, Maria Muñoz, Alessandro Crovetti, Juan Maria García-Casco, Riccardo Bozzi Use of NIRs for the assessment of meat quality traits in Iberian montanera pigs	Q05
17:45-18:00	Juan Manuel Cáceres Nevado, Ana Garrido Varo, Emiliano De Pedro Sanz, Dolores Pérez Marín  Discrimination between fresh and frozen-thawed Iberian pig m.  Longissimus dorsi by Near Infrared Reflectance Spectroscopy	Q06
18:00-18:15	Silvia Parrini, Valentina Becciolini, Alessandro Crovetti, Annalisa Romani, Silvia Urciuoli, Anna Acciaioli Effect of natural additives on physical and sensory characteristics of Cinta Senese sausage	Q07
18:15-18:30	Francisco J. Mesías, Carlos Díaz-Caro, Alberto Ortiz, Ahmed Elghannam, Susana García-Torres, Pilar Romero, Miguel Escribano, Paula Gaspar Consumers' preferences for sliced Iberian dry-cured ham in Spain: do production systems really matter?	Q08
19:00-20:00	WELCOME COCKTAIL (Venue: Camera di Commercio)	
	Thursday, 17/10/2019	
	Animal Breeding and Genetics	
Chairmen	Luca Fontanesi – Maria Muñoz	
09:00-9:30	MAIN LECTURE Francesco Tiezzi On the use of gut microbiome information in swine breeding	102
09:30-9:45	Cristina Óvilo, Maria Muñoz, Riccardo Bozzi, Juan García-Casco, Yolanda Núñez, Meta Čandek-Potokar, Anisa Ribani, Giuseppina Schiavo, Samuele Bovo, Silvia Tinarelli, Maurizio Gallo, Isabel Fernández, Luca Fontanesi and TREASURE CONSORTIUM  Genomic analysis and selection signatures in local European pig breeds	G01

09:45-10:00	Ana Heras-Molina, Consolación García-Contreras, Marta Vázquez-Gómez, Rita Benítez, Yolanda Núñez, Jaime Ballesteros, Jose Luis Pesántez-Pacheco, M.Victoria Sanz-Fernández, Susana Astiz, Beatriz Isabel, Antonio González-Bulnes, Cristina Óvilo Genotype, gender and interaction effects on piglet hypothalamic transcriptome	G02
10:00-10:15	Samuele Bovo, Giuseppina Schiavo, Anisa Ribani, Valerio Joe Utzeri, Giulia Moscatelli, Luca Fontanesi  Mining whole genome resequencing data from Italian pig breeds identifies selection signatures and putative causative mutations affecting phenotypic differences	G03
10:15-10:30	Adrián López-García, Rita Benítez, Yolanda Núñez, Emilio Gómez Izquierdo, Eduardo de Mercado, Juan García-Casco, Clemente López- Bote, Oscar González-Recio, Jordi Estellé, Cristina Óvilo Influence of breed and dietary energy source on gut microbiota composition in Iberian and Duroc pigs	G04
10:30-10:45	Martina Zappaterra, Paolo Zambonelli, Roberta Davoli Association study between SNPs in ACACA, ACLY, FASN, and SCD genes and fatty acid composition of backfat and muscle in Italian Large White pigs	G05
10:45-11:30	COFFEE BREAK	
11:30-11:45	Marie-José Mercat, Elias Zahlan, Matthias Petig, Herveline Lenoir, Pascal Cheval, Marjeta Čandek-Potokar, Martin Škrlep, Andrej Kastelic, Boris Lukic, João Nunes, Preciosa Pires, Bénédicte Lebret  An online database for growth, carcass and meat quality traits for future breeding programs in local pig breeds	G06
11:45-12:00	Anisa Ribani, Silvia Tinarelli, Valerio Joe Utzeri, Claudio Bovo, Stefania Dall'Olio, Maurizio Gallo, Luca Fontanesi  Application of DNA marker information to assure the integrity of autochthonous pig breed production chains	G07
12:00-12:15	Silvia Tinarelli, Maurizio Gallo, Luca Fontanesi  The Suis Project: actions for the conservation of Italian autochthonous pig breeds and for the improvement of sustainability	G08
12:15-12:30	Michela Ablondi, Paola Superchi, Valentino Beretti, Alberto Sabbioni  Exploring genetic diversity in Nero di Parma pig breed to develop  strategies for conservation and management	G09
12:30-12:45	Manolo Cappelloni, Maurizio Gallo, Roberto Steri, Luca Buttazzoni Genetic evaluation of the longevity of the traditional Italian Large White and Italian Landrace breeds	G10
12:45-13:00	Francesco Nen, Luciana Sartori, Manolo Cappelloni  Genetic programs for the preservation of Italian autochthonous pig  breeds: results and future prospects	G11
13:00-14:00	LUNCH	

	Socio Economics	
Chairmen	Marjeta Čandek-Potokar – Rui Charneca	
14:00-14:30	Main Lecture Ginevra Virginia Lombardi Pig farming in the Mediterranean area: social, environmental and economic aspects	103
14:30-14:45	Marjeta Čandek-Potokar, Zein Kallas, Urška Tomažin, Martin Škrlep, Nina Batorek-Lukač, Jose Maria Gil Consumer acceptance and market potential of products from Krškopolje pig	E01
14:45-15:00	Peter Balogh, Mónika Harangi-Rákos, Péter Kőmíves, Péter Lengyel, Dániel Fróna, Orsolya Nagy, Lajos Nagy Analysing producer opinions about traditional pig keeping in Hungary	E02
15:00-15:15	François Charrier, Mélanie Gallois, Oscar Maestrini, Stéphane Lugrezi, Fabien Casalta, François Casabianca  Experiencing new ways of designing pig disease management programs: emergence of a form of "middle management" of Aujeszky disease in Corsica	E03
15:15-15:30	Justine Faure, Ludovic Brossard, Florence Garcia-Launay, Marta Gil, Zein Kallas, José M. Gil  A qualitative and participative approach to analyse different strategies of valorisation of Noir de Bigorre pork chain	E04
15:30-15:45	Paula Gaspar, Natalia Carrillo, Francisco J. Mesías, Andrés Horrillo, Ahmed Elghannam, Miguel Escribano An insight about the Spanish quality standards for Iberian pig products by means of stakeholders' in-depth interviews	E05
20:00	GALA DINNER (Venue: Roof Garden Baglioni Hotel)	
	Friday, 18/10/2019	
	Farming System + Animal Health	
Chairmen	József Rátky – Oreste Franci	
09:00-9:30	MAIN LECTURE  Vicente Rodríguez-Estévez  Iberian pigs and dehesa: an agroforestry system	104
09:30-9:45	Martin Škrlep, Urška Tomažin, Nina Batorek Lukač, Marjeta Čandek- Potokar Quality of dry-cured ham from Krškopolje pig – comparison of organic and conventional housing system	F01

09:45-10:00	Mercedes Izquierdo, Nicolas Garrido, Francisco I. Hernández-García, Javier García-Gudiño, Miguel Angel Perez, Ana Isabel del Rosario Evaluation of muscle and fat deposition in three Iberian pig lines in two feeding systems by serial ultrasound scanning	F02
10:00-10:15	Francisco I. Hernández-García, Javier Matías, Maria López-Parra, Javier García-Gudiño, Veronica Cruz, Miguel Ángel Pérez, Carmen Barraso, Sonia Pardo, Nicolas Garrido, Mercedes Izquierdo  Effects of selenium-biofortified triticale feedstuff on meat quality and antioxidant capacity in extensive-raised Iberian pigs	F03
10:15-10:30	Perrine Devleeshouwer, Elie Chemel, Marie Gisclard, François Charrier, Oscar Maestrini  Slaughterhouses and pig farmers in Corsica: a systemic and geographical approach of a sanitary surveillance system	F04
10:30-10:45	Liane Dupon, Morgane Laval, Oscar Maestrini, François Charrier, Ferran Jori, Francois Casabianca  Using local knowledge to characterize Corsican pig farms practices for an epidemiological purpose	F05
10:45-11:30	COFFEE BREAK	
	Animal Breeding and Genetics	
Chairmen	Cristina Ovilo – Riccardo Bozzi	
Chairmen 11:30-11:45	Cristina Ovilo – Riccardo Bozzi  María Muñoz, Carmen Caraballo, Luis Silió, Carmen Rodríguez, Fernando Gómez, Fernando Sánchez, Juan María García-Casco  Association analyses for intramuscular fat content on purebred Iberian pigs using a costumed SNP panel from RNAseq data	G12
	María Muñoz, Carmen Caraballo, Luis Silió, Carmen Rodríguez, Fernando Gómez, Fernando Sánchez, Juan María García-Casco Association analyses for intramuscular fat content on purebred Iberian pigs using a costumed SNP panel from RNAseq data Roberta Davoli, Martina Zappaterra, Domenico Pietro Lo Fiego, Paolo Zambonelli Green Charcuterie project: effects of pig diets added with linseed and antioxidants on muscle gene expression and identification of differentially expressed genes	G12
11:30-11:45	María Muñoz, Carmen Caraballo, Luis Silió, Carmen Rodríguez, Fernando Gómez, Fernando Sánchez, Juan María García-Casco Association analyses for intramuscular fat content on purebred Iberian pigs using a costumed SNP panel from RNAseq data Roberta Davoli, Martina Zappaterra, Domenico Pietro Lo Fiego, Paolo Zambonelli Green Charcuterie project: effects of pig diets added with linseed and antioxidants on muscle gene expression and identification of	

13:00-14:00	LUNCH	
	Nutrition and Reproduction	
Chairmen	Rosa Nieto – Carolina Pugliese	
14:00-14:30	MAIN LECTURE  Rosa Nieto  Iberian pig as a model to study protein and lipid metabolism in fatty  pigs	105
14:30-14:45	Wendy M. Rauw, Juan M. García Casco, Fernando Gómez Carballar, Luis Alberto García Cortés, Eduardo de la Serna Fito, Patricia Palma Granados, Miguel A. Fernández Barroso, Luis Gomez-Raya Relationship between feed efficiency and meat quality in Iberian pigs	N01
14:45-15:00	Luigi Gallo, Stefano Schiavon, Celio Paolo Sasso, Paolo Carnier  Performance, carcass and ham traits of heavy pigs managed on different targets for age and weight at slaughter	N02
15:00-15:15	Ignacio Fernández-Fígares, Luis Lara, Manuel Lachica Betaine increases portal appearance of branched chain amino acids in Iberian pigs	N03
15:15-15:30	Antonio González-Bulnes, Susana Astiz, Maria Victoria Sanz-Fernández, Consolación García-Contreras, Ana Heras-Molina, Jose Luis Pesántez-Pacheco, Marta Vázquez-Gómez  The relative roles of birth-weight and postnatal diet on patterns of growth and fattening in Iberian pigs	N04
15:30-15:45	Ana Heras-Molina, Marta Vázquez-Gómez, Consolación García-Contreras, Victoria Sanz-Fernández, Laura Torres-Rovira, Jose Segura-Plaza, Jose-Luis Pesántez-Pacheco, Susana Astiz, Cristina Óvilo, Antonio González-Bulnes, Beatriz Isabel  Effect of maternal antioxidant and omega-3 supplementation on the subcutaneous fatty acid profile of the Iberian offspring	N05
15:45-16:00	Manuel Lachica, Ignacio Fernández-Fígares, Isabel Seiquer, Luis Lara, Fernando Sánchez-Esquiliche, Fernando Gómez-Carballar, José María Pariente, Rosa Nieto Performance of lactating Iberian sows fed diets of different protein concentration	N06
	POSTER SESSION	
Topic		
Animal healt	Cristina Tabuc, Mihaela Dumitru, Mihaela Hăbeanu  Effects of a bioadditive based on Lactobacillus strain in weaning piglets	P01

Farming system	Carlos Díaz-Caro, Alberto Ortiz, Francisco J. Mesías, Pilar Romero, Miguel Escribano, Paula Gaspar The cost of Iberian pig in extensive conditions: a case study of different marketed products according to Spanish regulations	P02
Farming system	António do Rosário Oliveira, Manuel Joaquim Marques Patanita, Mariana Augusta Casadinho Parrinha Duarte Regato, Maria de Fátima Nunes de Carvalho, Maria Adelaide Araújo Almeida, Carlos Manuel Marques Ribeiro, Anabela Cândida Ramalho Durão, Nuno Manuel Nobre de Brito Faustino, Pedro Camacho Autochthonous Portuguese Alentejo Local Pig Breed (Sus ibericus, SANSON, 1901): Preliminary Scientific Notula II	P03
Farming system	Javier García-Gudiño, Isabel Blanco-Penedo, Alessandra Monteiro, Sandrine Espagnol, Florence Garcia-Launay Environmental impacts of contrasted Iberian pig production systems	P04
Farming system	Javier García-Gudiño, Mercedes Izquierdo, Javier Matías, Veronica Cruz, Miguel Á ngel Pérez, Sonia Pardo, Francisco I. Hernández-García Optimization of Iberian pig extensive systems during the growing period: use of alternative protein sources through grazing	P05
Farming system	Elena González, María Muñoz, Miguel Angel Fernández- Barroso, Carmen Caraballo, Juan M. García-Casco Growth description of Iberian x Duroc crossbred pig through the evaluation of nonlinear mathematical models	P06
Farming system	Elena González, Alberto Ortiz, Susana García-Torres, María Cabeza de Vaca, David Tejerina Relationship between several live animal measurements and carcass quality in Iberian pigs	P07
Farming system	Eleonora Nannoni, Domenico Ventrella, Andrea Antonelli, Alessandro Valicelli, Giovanna Martelli, Luca Sardi Group housing of sows: effects of season and day of grouping on sow welfare and reproductive performance	P08
Farming system	Sebastiano Porcu, Giovanni Piras, Francesco Nuvoli, Gianni Battacone, Carlo Diaferia, Gian Marco Saba, Giuliano Patteri Woodland and livestock systems in inland areas of Sardinia: the case of the native pig breed	P09
Farming system	Dubravko Škorput, Danijel Karolyi, Krešimir Salajpal, Sven Menčik, Vedran Klišanić, Željko Mahnet, Zoran Luković <b>Production traits of Banija spotted pig</b>	P10
Farming system	Joan Tibau, Raquel Quintanilla, Joel Gonzalez, Neus Torrentó, Pere Antoni Company, Josep Cifre, Jaume Jaume Effect of feed restriction and sex on growth performances and carcass composition in "Porc Negre Mallorqui" pig breed	P11

Farming system	Francesco Sirtori, Silvia Parrini, Chiara Aquilani, Oreste Franci, Carolina Pugliese, Anna Acciaioli, Riccardo Bozzi Forage chain management in rearing system of Cinta Senese	P12
Animal Breeding and Genetics	André Albuquerque, Cristina Óvilo, Yolanda Núñez, Rita Benítez, Adrián Lopéz-García, Jaime Ballesteros, Fabián García, Marta Laranjo, Rui Charneca, José Manuel Martins Identification of differentially expressed key genes of Longissimus lumborum samples from Portuguese Alentejano and Bísaro local pig breeds	P13
Animal Breeding and Genetics	Miguel Ángel Fernández-Barroso, Carmen Caraballo, Fernando Gómez, Yolanda Núñez, Juan María García-Casco, María Muñoz SNP association analyses for myoglobin content and water holding capacity in a purebred Iberian pig population	P14
Animal Breeding and Genetics	Kristina Gvozdanović, Ivona Djurkin Kušec, Vladimir Margeta, Žarko Radišić, Goran Kušec Carcass and meat quality of purebred Black Slavonian pigs and crossbreds with Duroc	P15
Animal Breeding and Genetics	Polona Margeta, Vladimir Margeta  Changes in the genetic status of Crna slavonska pig breed over 15 years	P16
Animal Breeding and Genetics	Yolanda Núñez, Radović Čedomir, Savić Radomir, Marjeta Čandek-Potokar, Rita Benítez, Radojković Dragan, Lukić Miloš, Gogić Marija, Luca Fontanesi, Cristina Óvilo Muscle transcriptome in Mangalitsa and Moravka pigs	P17
Animal Breeding and Genetics	Dubravko Škorput, Minja Zorc, Kristina Gvozdanović, Polonca Margeta, Zoran Luković, Danijel Karolyi, Vedran Klišanić, Sven Menčik, Peter Dovč, Krešimir Salajpal Optimal contribution selection: a tool for sustainable management of Banija spotted pig	P18
Animal Breeding and Genetics	Masaaki Taniguchi, Aisaku Arakawa, Shinya Ishihara, Quang Minh Luu, Doan Lan Pham, Van Ba Nguyen, Nguyen Cong Dinh, Pham Hai Ninh, Ngo Thi Kim Cuc, Kazuhiro Kikuchu, Satoshi Mikawa Genetic analysis on Vietnamese native pigs: variations of coat color and porcine endogenous retrovirus genes	P19
Animal Breeding and Genetics	Miguel Angel Fernández-Barroso, Patricia Palma-Granados, Carmen Caraballo, Fernando Gómez, María Muñoz, Juan María García-Casco  Technical validation of longissimus dorsi transcriptomic expression on purebred Iberian pigs divergent for meat tenderness from a RNAseq experiment	P20
Meat Quality and Food Technology	Ramón Cava, Guadalupe Lavado, Nieves Higuero, Irene Montero, Luis Ladero Lactic acid and glycogen contents, pH and meat quality from Iberian pigs raised on free-range and fed on acorn and grass: early, middle and late "montanera"	P21

Meat Quality and Food Technology	Carlos Díaz-Caro, Francisco J. Mesías, Alberto Ortiz, Ahmed Elghannam, David Tejerina, Andrés Horrillo, Eva Crespo, Paula Gaspar Modeling preference heterogeneity and willingness to pay for Iberian dry-cured ham	P22
Meat Quality and Food Technology	José Manuel Martins, André Albuquerque, Rita Fialho, José Neves, Amadeu Freitas, José Tirapicos Nunes, Rui Charneca Alentejano and Bísaro pigs and their crosses: genotype effect on loin traits	P23
Meat Quality and Food Technology	Silvina Ferro Palma, Maria João Carvalho, António Floro, María Jesús Martín Mateos, Jesús García-Parra Effects of high pressure on properties of meat products	P24
Meat Quality and Food Technology	Luca Sardi, Andrea Rossi, Enrica Gorlani, Andrea Bertolini, Giulia Rubini, Ruben Cantagallo, Giovanna Martelli, Eleonora Nannoni Can raw thighs classified as E be suitable for the production of PDO hams?	P25
Meat Quality and Food Technology	Radomir Savić, Čedomir Radović, Dragan Radojković, Nenad Parunović, Marija Gogić, Bénédicte Lebret, Marjeta Čandek- Potokar Effect of immunocastration on chemical content and fatty acid composition of fat tissue of Mangalitsa pigs	P26
Meat Quality and Food Technology	David Tejerina, Rebeca Contador, Susana García-Torres, María Cabeza de Vaca, Alberto Ortiz  Potential use of near infrared reflectance spectroscopy (NIRS) to predict the commercial categories of pre-sliced and packaged Iberian dry-cured ham	P27
Meat Quality and Food Technology	Marta Vázquez-Gómez, Consolación García-Contreras, Laura Torres-Rovira, José Luis Pesantez-Pacheco, María Victoria Sanz-Fernández, Ana Heras-Molina, Susana Astiz, Cristina Óvilo, Antonio González-Bulnes, Beatriz Isabel Changes in tissue fatty acid composition during postnatal development in Iberian pigs	P28
Meat Quality and Food Technology	Silvia Parrini, Christos Dadousis, Danijel Karolyi, José Manuel Martins, Juan Maria Garcia-Gasco, Nuria Panella-Riera, Rosa Nieto, Matthias Petig, Violeta Razmaite, Ivona Djurkin Kušec, José Pedro Araujo, Meta Candek-Potokar, Bénédicte Lebret, Claudio Cipolat Gotet, Riccardo Bozzi Tracing autochthonous pig breeds with meat near-infrared spectra data	P29
Meat Quality and Food Technology	Chiara Aquilani, Francesco Sirtori, Corrado Dimauro, Riccardo Bozzi, Oreste Franci, Luca Calamai, Anna Acciaioli, Antonio Pezzati, Carolina Pugliese  Evolution of the volatile compounds along curing in Semimembranosus muscle of the Toscano ham	P30
Meat Quality and Food Technology	Maria José Cardador, Andrés Martín-Gómez, David Saavedra, Vicente Rodríguez-Estévez, Lourdes Arce	P31

	Gas chromatography-ion mobility spectrometry: a portable instrument to ensure Iberian ham traceability during its processing	
Nutrition and Reproduction	Elena González, José Luis Noguera, Noelia Ibáñez-Escriche, María Jesús García-Santana, Miguel Ángel Fernández- Barroso, Juan Florencio Tejeda Fatty acid composition, lipogenic enzyme activities and increased thickness of subcutaneous adipose tissue of two lines of Iberian pigs subjected to three types of feed during the fattening period	P32
Nutrition and Reproduction	Mihaela Hăbeanu, Nicoleta Aurelia Lefter, Anca Gheorghe, Arabela Untea, Mariana Ropotă, Cristina Tabuc Effects of n-3 polyunsaturated fatty acids rich diet on nitrogen metabolism in growing barrows	P33
Nutrition and Reproduction	Nicoleta Aurelia Lefter, Mihaela Hăbeanu, Anca Gheorghe, Lavinia Idriceanu Effects of millet grain diet on growth performance and certain plasma parameters of growing pigs	P34
Nutrition and Reproduction	Isabel Seiquer, Ana Haro, Patricia Palma-Granados, Luis Lara, José F. Aguilera, Rosa Nieto Performance of growing Iberian pigs fed diets of different Ca and P concentration	P35
Nutrition and Reproduction	Consolación Garcia-Contreras, Marta Vazquez-Gomez, Ana Heras-Molina, José Luis Pesantez, Teresa Encinas, Susana Astiz, Laura Torres-Rovira, Beatriz Isabel, Cristina Ovilo, Antonio Gonzalez-Bulnes Effects of Maternal Hydroxytyrosol Supplementation on Fatty Acids Composition of Iberian Swine Fetuses	P36

X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

### SESSION 1

### MEAT QUALITY AND FOOD TECHNOLOGY

### MAIN LECTURE

### Distinctive characteristics of Iberian pig meat

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Traditional production of Iberian pigs finished on acorn and grass in a Mediterranean forest (Dehesa) has a long history with references long before the Roman domination of the Iberian Peninsula. Main peculiarities of this production system include: 1) BIOLOGICAL PECULIARITIES OF THE PIGS, outstanding among them, i.-High proportion of red aerobic muscle fibers which determines a red color of meat, but also a low muscular mass proportion and also some peculiar biochemical properties of meat in comparison to improved pig genotypes (including postmortem pH drop and ultimate pH), ii.-High adipogenic and low protein accretion (approx, 70-80 g/d) potential, thus determining a high intramuscular fat content, and a wide distribution of tiny lipids droplets which produces a peculiar marbling, iii.-Well adaptation to periods of feed restriction (thrifty genotype) and ad libitum very high feed intake (including compensatory growth), iv.-Good ability to feed foraging and exercise (with interesting metabolic adaptations), iv.- Intake of feed materials directly from the field, thus containing active molecules, including a high variety of antioxidants, v.- High content of monounsaturated fatty acids derived from acorn intake, but also from an enhanced metabolic ability to endogenously unsaturated fatty acids in delta-9 position, vi.- Peculiar distribution of fatty acids within the triglyceride molecule (with a lower concentration of palmitic acid in the sn2 position in comparison to other pig genotypes). These peculiarities requires a complete different production systems from those used in conventional pigs (eg different feeding protocols, raw material, slaughter houses, farm desing, equipment for handling, transport, slaughter, and so on). 2.- DIFFERENTIATED CHARACTERISTICS OF MEAT PRODUCTS (particularly dry cured ham), which is identified by the market as something unique and of high quality, thus reaching a higher price in comparison to conventional pigs. Moreover, fatty acid composition and distribution within the triglyceride, as well as high and unique natural antioxidant concentration has been associated to health benefits in comparison to other pork products. 3) ENVIRONMENTAL PECULIARITIES. Traditional production was done extensively throughout the whole period, with a characteristic finish in Dehesa in autumn, thus having fully access to unlignified grass and newly ripen acorns. Production cycle of pigs is closely linked to that of ruminant animals, which passes sequentially in the Dehesa to eat lignified grass, hay, and other residuals, before the pig re free into the Dehesa. Thus, the Iberian pig production system is main responsible for a combined and sustainable production system support survival of some other production systems, the maintenance of the traditional Mediterranean forest (including the control of summer forest fires) and ultimately to the fixation of rural population. Outdoor production of Iberian pigs reaches a high standard of animal welfare (over 1 hectare per pig), which is highly accepted for the market. 4) SOCIAL PECULIARITIES. Traditional Iberian pig production has survived modern practices of swine production which almost completely supplanted all swine production systems in Europe from the mid XX century not only because of its peculiarities and quality, but also because Dehesa forest only existed in under developed regions of Spain, located peripherical, far from ports and large markets, with a deficient trains communication system and a very low income. In other words, regions where intensive production could not compete with some other areas closer to the ports and communication systems, or near big cities.

X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

Afore mentioned characteristic has allowed the development of a niche market aimed to high income consumer and specialized gourmet sector, thus supporting the survival of the Iberian pig and la Dehesa forest. However, real social impact of this production system have severe limitations, as total number of Iberian pigs fed on acorn and grass cannot surpass 500.000 pigs/year due to limitation of Dehesa forest. Production of this forest cannot be increased due to the very long production of evergreen oaks (which require more than 50 years to be productive). Moreover, production of traditional Iberian pigs is seasonal (slaughter taking place only during 3-4 winter months) which make the specialized slaughter houses and other facilities as well as specialized professional labor force difficult to maintain. This open the discussion for a new concept of sustainable Iberian pig production, in which modern practices are designed and developed to maintain high standard of biodiversity, sustainability, animal welfare standards and peculiar quality characteristics, thus allowing the production of a higher number of pigs feed with mixed diets and aimed to a wider market. Adequate traceability and labelling are essential to clearly differentiate products.

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ABSTRACT

## Flavour generation in dry cured loins as affected by paprika addition and reduced nitrite/nitrate ingoing amounts

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The presence of spices in meat processing may produce a high impact in sensory characteristics. Furthermore, they can be a source of nitrate as occurs in the use of paprika for dry cured loin manufacture. Paprika antioxidant properties affect dry loin colour and flavour characteristics. Also, the microbial load of paprika may contribute to the ripening process and impact flavour development. The demand for healthier meat products with natural ingredients and reduced ingoing amounts of curing agents has driven the need to elucidate the contribution of spices. Therefore, the objective of our study was to investigate the mechanisms of aroma formation in dry cured loin processed with reduced content of nitrifying agents and using paprika as an external source of nitrate. Two groups of dry cured loins were manufactured with and without paprika as seasoning. In each group, three treatments with different curing agent contents were applied: Control C (150 ppm of sodium nitrite and potassium nitrate), 25% R25 (113 ppm of each curing agent), and 50% R50 reduction (75 ppm of each curing agent). The loins were ripened for 43 days until a weight loss of 40% was reached. Physic-chemical analysis (composition, nitrate and nitrite residual content), free amino acid content by GC-FID and volatile analysis by SPME-GC-MS were done on the ripened loins. The data was analysed using the linear mixed model, including nitrite/nitrate contents and paprika addition as fixed effects and the loin as the experimental unit.

Results revealed that the nitrate residual concentration was affected by the different ingoing amounts of curing agents and paprika added. Paprika contributed to 17-20 ppm sodium nitrate, in accordance with paprika nitrate content. Free amino acids content as precursors of volatile compounds in dry cured loins was significantly higher (P < 0.01) in loins manufactured with paprika. However, only a small percentage of free amino acids seemed to be directly originated from paprika, but proteolytic activities from microorganisms present in paprika would be partially responsible for this increase. The production of volatile compounds in the dry cured loins was affected mainly by paprika addition and, in lower amounts by nitrite reduction. The antioxidant effect produced by paprika in dry cured loins was in agreement with the lowest significant abundance of detected lipid oxidation volatile compounds. Furthermore, paprika addition increased volatiles originated from the catabolism of amino acids by bacteria metabolism. In conclusion, flavour generation would be the result of the synergistic effect of paprika and ingoing nitrite/nitrate amounts, as well as, the contribution of the proteolytic activity from selected microbial groups, the catabolism of the free amino acids and paprika antioxidant effect.

#### Acknowledgemen

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### SESSION 1

### MEAT QUALITY AND FOOD TECHNOLOGY

### ORAL COMMUNICATIONS

16-18 October 2019
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ABSTRACT

## Effect of animal's diet, processing method and muscle type on quality traits of dry-cured hams produced from Turopolje pigs

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Turopolie pig (TP) is a local Croatian pig breed which nearly extinct in the second half of the 20<sup>th</sup> century. Currently, despite the state support, the TP breed is still endangered and to self-sustain it a new conservation strategy, based on its traditional production system and products with an enhanced added value, is needed. So far, a little information is available on quality traits of TP meat products, including the effects of locally available feeding resources or processing innovations aimed at reducing the potentially undesirable compounds. Hence, the objective of present study was to examine the effect of animal's diet (acorn supplementation or conventional), processing method (standard, less salting or less smoking) and muscle type (m. biceps femoris - BF or m. semimembranosus – SM) on quality traits of dry-cured hams produced from TP breed. Tested hams (n=30) were produced by the same basic process (dressing, dry salting, smoking and drying) and were equally distributed by investigated effects. Sampling of ham, caudal part with BF and SM muscles, for chemical analysis was performed when products were about 15 months old. The quality traits of ripe ham, including processing loss percentage, pH and water activity (aw) values, salt (NaCl), moisture, fat and protein content, proteolysis index, CIE  $L^*$ ,  $a^*$ ,  $b^*$  colour and texture profile analysis (TPA), were determined. Differences between effects were analysed by the general linear model (GLM) procedure of SAS 9.4 at an alpha level of 0.05. Results showed that hams from acorn supplementation had lost less weight and were higher in moisture and lower in proteins, with a higher proteolysis index and colour  $b^*$ . The method of processing affected only less salted hams which had a lighter  $(L^*)$  muscle colour and were lower in NaCl content but with a higher aw and pH values and more pronounced proteolysis. Finally, a strong effect of muscle type was observed: compared to SM, BF had lower protein content but was higher in moisture and NaCl content, proteolysis index and  $L^*$ ,  $a^*$ ,  $b^*$  colours, and exhibited softer texture with lower values of hardness, gumminess and chewiness. In conclusion, this study indicated a notable effects of animal's diet, processing method and muscle location on dry-cured hams quality, most prominently in terms of higher moisture retention and processing yield in "acorn-fed" hams, the impaired quality and conservation parameters in hams with salt reduction, and lower dehydration, more intensive colour, softer texture and higher saltiness of BF muscle.

### Acknowledgement

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### Quality of pork from European local pig breeds: analytical study on the average and variability of sensory, technological and nutritional traits within and between breeds - TREASURE project

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X International Symposium of Mediterranean Pig

Within TREASURE project the sensory, technological and nutritional properties of fresh meat and traditional pork products issued from a variety of European local breeds were characterised. Using common physicochemical, biochemical and sensory indicators and methodologies, the quality of fresh loin, green ham, and back fat from 14 local breeds issued from 16 experiments (Alenteiana: ALT, Bísara: BIS, Cinta Senese: CSE, Crna slavonska: CSL, Gascon: GAS, Iberíco: IBR, Krškopolje: KRP, Lietuvos baltosios: LBA, Lithuanian indigenous wattle: LVI, Mangulica: MAN, Moravka: MOR, Porc negre mallorquí: PNM, Schwäbisch-Hällisches: SWH, Turopoliska svinja: TUR) was determined by project partners. A descriptive analysis on quality variability was undertaken based on mean and 95% confidence interval (=1.96\*standard deviation) values collected per breed and tissue for each quality trait. Results highlighted high variability of intrinsic quality of meat and products, both between and within breeds, especially for intramuscular fat content (IMF) of loin and ham, fatty acid (FA) profile of back fat, loin and ham muscles, lightness (L\*) of loin and ham muscles, drip loss, cooking loss and shear force of loin. Lowest average loin IMF was found in GAS, LBA, LVI, SWH and TUR pigs (2.1 to 2.6%), whereas eight breeds exhibited over 6% IMF (10.1% in MAN and 8.1% in MOR). Variability of IMF content also greatly differed within breeds. In all tissues, mono-unsaturated were the most abundant FA group, representing between 43-45% (MOR, MAN, KRP) and 60% (IBR) in back fat, this latter being associated with Montanera finishing system. Saturated FA varied between 29 and 46%, whereas poly-unsaturated (PUFA) varied between 7% (GAS, PNM) and 15% (KRP). Within PUFA, the high variation in n-6/n-3 ratio (1.5 in MAN, MOR up to 15 in PNM) may be related to feed composition differences between experiments. Average loin ultimate pH varied between 5.43 (LBA, LVI) and 5.97 (TUR) but may partly be related to pre-slaughter handling conditions. Loin L\* was the lowest (< 45) in ALT, GAS, IBR, PNM and TUR pigs, and the highest in LBA, LVI, MAN and MOR (around 55), with high variability in most breeds. Even though some results should be interpreted with caution due to low number of animals in some breeds, the use of common indicators and methodologies to analyse meat quality allow drawing a first picture of the variability of intrinsic qualities of pork from these genetic resources.

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ABSTRACT

### Apulo-Calabrese breed: Preslaughter-stress and meat characteristics

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Apulo-Calabrese is a local pig breed from southern Italy. This breed is well adaptable to different production systems and produces meat with quality characteristics suitable for the production of four Protected Designation of Origin salami. Stress is an inevitable consequence of pre-slaughter handling that can have adverse effects on haematological and pork quality traits. However, not much is known on the effects of pre-slaughter stress on the welfare and the meat quality traits of local breeds such as Apulo-Calabrese. We investigate the effect of stress on the welfare and meat quality traits of Apulo-Calabrese in comparison with crossbreed [Duroc x (Landrace and Large White) pigs. Fifty one Apulo-Calabrese and fifty two crossbreed pigs reared in the same conventional farm were transported for approximately one hour to a local processing plant. All the animals were blood sampled five days before transport and at exsanguination for the analysis of blood stress parameters. Apulo-Calabrese pigs were slaughtered at 135 kg live weight while crossbreed pigs were slaughtered at 155 kg live weight. Meat quality traits such as pH, color, drip loss, cooking loss, Warner-Bratzler shear force and intramuscular fat (IMF) were assessed on the longissimus thoracis (LT) muscle. In addition, water status was assessed from LT samples using transverse relaxation time (T<sub>i</sub>), weighted signals registered by Time Domain Nuclear Magnetic Resonance (TD-NMR) and fatty acid composition was determined by Folch method. Data were analyzed using mixed models and principal component analysis. Although a large number of the measured blood parameters were within the normal physiological range, Apulo-Calabrese pigs showed the highest value of exsanguination lactate when compared with crossbreeds (P < 0.0001). Meat quality traits were similar for both genetic types. However, significantly (P < 0.05) higher a\* and lower L\* coordinates were found in the samples of Apulo-Calabrese which showed meat with a deeper red color than crossbreeds. Results from the TD-NMR showed higher percentage (P =0.004) of extra-myofibrillar water in the samples of Apulo-Calabrese which was in agreement with the higher values of cooking loss found in this breed at 24 and 72 hours postmortem. The content of IMF was higher in Apulo-Calabrese (P = 0.08), which showed also significantly higher contents of heptadecenoic acid (P < 0.0001), myristic (P = 0.03), arachidic (P = 0.04), myristoleic (P = 0.004), palmitoleic (P = 0.01) and gondoic (P = 0.01) acids. These data provided insight on the effects of pre-slaughter stress on the well-being and meat quality traits of Apulo-Calabrese pig.

### Acknowledgement

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### Use of NIRs for the assessment of meat quality traits in Iberian montanera pigs

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X International Symposium of Mediterranean Pig

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Iberian pig rearing system is characterized by a montanera final period based on acorns and grass as feed. This traditional system combined to the genetic characteristics of the breed can influence meat quality. Determination of meat characteristics by a rapid and no destructive technique as Near Infrared Spectroscopy can be useful in order to highlight the qualitative proprieties of fresh meat. The object of this study was to investigate the FT-NIRs capacity to estimate qualitative characteristic of Iberian pigs intact fresh meat. Purebred Iberian castrated male pigs (n=287) slaughtered at 17 months of age and at an average weight of 165 kg, were used in the present study. From each carcass, samples of *Longissimus dorsi* muscle were removed, vacuum-packed and stored at -20°C until analysis. Traditional analysis was applied in order to determine tenderness (with Warner-Bratzler Shear Force and Texture Profile Analysis), myoglobin content, water holding capacity and color. For each sample, two aliquots of intact meat were scanned using FT-NIRs Antaris TM II Analyzer. Mathematical pre-treatments (Multiplicative Scatter Correction, Standard Normal Variate, 1st and 2nd derivate) were applied and partial least square regression was used on the average spectrum. Results were evaluated in terms of coefficient of regression and root mean square errors in calibration ( $R^2$ -RMSE) and validation ( $R_v^2$ -RMSEV), as well as in terms of residual prediction deviation (RPD). Equations were developed in full spectral range because optimisation of the spectral range did not improve the results. Calibration results obtained R<sup>2</sup> values between 0.76 and 0.79 for colour parameters and RMSE between 0.7 and 1.9. Myoglobin content achieved a R<sup>2</sup> value of 0.62 whereas water holding capacity of 0.73. Warner Bratzler Shear Force obtained better calibration results than Texture Profile Analysis (0.79 and 0.70 respectively). R<sup>2</sup> were lower and RMSEV were higher in validation compared to calibration. Furthermore, RPD in calibration was always higher than 1.5, except for myoglobin, indicating that the use of NIRS on intact fresh meat have the potential to be used for classifying the results into different classes.

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### Discrimination between fresh and frozen-thawed Iberian pig m. Longissimus dorsi by near infrared reflectance spectroscopy

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In recent years, interest in meat authenticity has increased. Many consumers are concerned about the meat they eat, and accurate labelling is important to inform consumer choice. The ability of near-infrared (NIR) spectroscopy to authenticate fresh and frozen-thawed Iberian pig Longissimus dorsi was tested using partial least squares discriminant analysis (PLS-DA). A total of 75 samples, which come from animals reared during 2017 and 2018 and slaughtered with an average weight of 150 kg. These samples were selected for this purpose and analysed using a portable, handheld, linear variable filter system (LVF) instrument MicroNIR Onsite Lite (spectral range 908.1-1676.2 nm), working in reflectance. Firstly, fresh samples (N=45) were measured immediately after purchased. Secondly, frozen-thawed samples (N=30) were measured after frozen at minus 20 °C and stored at the same temperature for more than 90 days (3-6 months), then thawed in the refrigeration at 4 °C for 18 hours. Differences in spectra data of fresh and frozen-thawed meat samples were found. The best models were obtained using Mean Center transformation of spectra data, which correctly classified 100% of the samples from calibration set. The obtained recognition ratio for validation set was 100% for fresh and frozen-thawed meat. From the obtained results it can be concluded that NIR spectroscopy and PLS-DA have a great potential for discriminating fresh from frozen-thawed minced pig meat (Longissimus dorsi).

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Florence. Italy ABSTRACT

## Effect of natural additives on physical and sensory characteristics of Cinta Senese sausage

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Cinta Senese is a local pig breed typical of Tuscany, and traditionally its meat is processed in order to produce dry cured products. The use of curing agents as nitrites and nitrates is effective to increase the shelf-life of products and to stabilise their organoleptic properties, however their intake may represent a risk for human health. The aim of the study was to test the use of natural antioxidants as curing agents and to determine their effects both on the physical parameters and on the sensory profile of Cinta Senese sausages (würstels). Meat was obtained from two groups of stall-fed pigs, in order to have two replicates of the study design. Each meat batch was divided in two parts: one (NN) was processed using nitrites and nitrates as curing agents; the other (NA) was processed using natural extracts rich in antioxidant and antimicrobial compounds as chestnut hydrolysable tannins, tannins condensed from grape seeds, hydroxytyrosol and oleuropein from Olea europaea. The mixture of natural extracts was characterized by HPLC/DAD/MS and the antioxidant and antiradical capacity was evaluated. Vacuum-packed sausages were stored for 60 days at +4°C. Physical and microbiological parameters were determined at different storage periods: 7, 30 and 60 days. Colour (lightness L\*, redness a\* and yellowness b\*) was measured with a Minolta® spectrophotometer. Samples were subjected to Texture Profile Analysis in order to assess hardness, chewiness, springiness and cohesiveness. Sensory attributes were determined using a ten-member trained panel and expressed in a continuous scale from 1 to 10. Data were analysed with Linear Mixed Models (R 3.4.1). Storage period affected redness and yellowness (p < 0.01), but only in NA. All colour parameters differed between NN and NA (p < 0.01): sausages cured with NN showed greater lightness and redness, but lower yellowness. All texture parameters resulted higher in NN (p < 0.001), except for the springiness. The panellists experienced in NN sausages greater colour homogeneity, uniformity, hardness and lightness (p < 0.01); in NA sausages, they perceived higher odour intensity and marked flavours as acid and tannic (p < 0.01). The NN sausages displayed greater global acceptability. The natural antioxidants mixture showed an effective antimicrobial activity, however further studies are needed to improve the organoleptic profile of NA products and, thus, their liking.

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Florence, Italy ABSTRACT

## Consumers' preferences for sliced Iberian dry-cured ham in Spain: do production systems really matter?

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Spanish consumers have a strong preference for Iberian meat products -especially dry-cured hamas they perceive them to be of extra sensorial and nutritional quality. The production of these meat products depends on multiple variables, such as genetics (pure Iberian, Iberian crossed with other breeds...), livestock production systems (extensive or with various levels of intensification) and, above all, the feed provided (acorn, pastures and/or feedstuffs) etc. The aim of this paper is to study the preferences of Spanish consumers for the various types of sliced Iberian dry-cured ham, analysing whether they are willing to pay the premium required by the highest-quality products, which would allow producers to match production and demand. The methodological approach combined a sensory analysis and a choice-based conjoint experiment. The use of both tools allowed not only to examine the attributes that could shape consumer purchasing decisions (information about origin or production system, packaging format and price,) but also the sensory attributes (odour, taste,...) that are assessed after purchase, which to a great extent, may be responsible for the repetition of the choice. Data were obtained through tasting sessions in Extremadura (SW of Spain). Participants were required to evaluate the attributes of Iberian dry-cured ham -overall appearance, odour, texture, taste and overall liking- using a linear scale of 1 (dislike extremely) to 5 (like extremely). After the sensory test, participants were given detailed information regarding the different categories of Iberian dry-cured ham and were asked to complete a choice experiment task. Sensory analysis revealed that consumers prefer dry-cured hams obtained from pigs raised in the traditional "montanera" system, where they are mainly fed on acorns and pasture (vs. feeding on concentrates in the conventional system). Meanwhile, although the purity of the Iberian breed is usually taken as one of the defining aspects for top-quality Iberian dry-cured hams, this attribute has not been appreciated in this study. These findings were confirmed by the results of the choice experiment -which could be associated with a pre-purchase situation- and where consumers indicated their highest preference for montanera hams, being the "type of feed" the most important attribute and the "% Iberian breed" the one with the lowest importance. Choice experiment has also proven to be a useful tool to determine the economic value that consumers allocate to the different levels of the attributes, which could be used in future marketing strategies in the Iberian sector.

### Acknowledgements

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### **SESSION 2**

### ANIMAL BREEDING AND GENETICS

### MAIN LECTURE

Florence, Italy ABSTRACT

### On the use of gut microbiota information in swine breeding

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With the advent of cost-effective sequencing, the study of pig gut microbiome has gained interest. The gut microbiome is the ensemble of species that co-exist inside the intestine of animal species as hosts. It can influence the host's physiological and immunological processes and, ultimately, contribute to the determine the phenotype. In humans as well as pigs, changes in gut microbiota composition can heavily affect health status and production performance of the host (Guevarra *et al.*, 2018).

In the era of precision agriculture, the gut microbiome can provide valuable information, particularly when coupled with economically relevant phenotypes. While there is evidence that the metagenome can be influenced and shaped by external interventions (management, diet formulation, feed supplementation), animal (swine) breeders aim at exploiting the variability in gut microbiome to select the host. Given that the exploitation of microbiome information in swine (and livestock) breeding in general is a recent concept, several preliminary steps are necessary to understand its potential use. Recent studies have characterized the impact of non-altered gut microbiome characteristics in relation to growth and carcass traits (Park *et al.*, 2014), lipid metabolism (Xiao *et al.*, 2018) and fiber utilization (Cheng *et al.*, 2018). Authors have also found that the species are not independent in their abundance in the intestine, and defined enterotypes that can be linked to growth traits (Ramayo-Caldas *et al.*, 2016). The diversity on the microbial profile has also been linked to growth and tissue deposition measures (Lu *et al.*, 2018).

With the increasing number of studies reporting the impact of microbiome on phenotypes, Difford *et al.* (2016) reported the term 'microbiability' for the first time. This measure was suggested as a way to include the overall microbial composition as part of phenotypic variation, without being specific on the taxonomic dissection of the microbiome itself. A microbial "relationship" matrix obtained from the taxonomic abundance for each individual can be constructed, in a similar fashion that host genotypes can be used to build a genomic relationship matrix. The microbiome relationship matrix can then be fitted into a standard linear mixed model, and the proportion of phenotypic variance absorbed by such effect will be expressed as the microbiability of the trait. In other words, the linear model aims at dissecting the variance in order to find that part that depends directly on the microbiome: with a given change in microbiome composition, a respective change in phenotype is expected. Estimates of microbiability in swine are still scarce in the literature. Camarinha-Silva *et al.* (2017) and Lu *et al.* (2018) identified a sizable microbiability component in pigs for several growth traits.

A pitfall of the microbiability concept is that it ignores the contribution that the host genome can have on microbiome composition, which could, in turn, be reflected on the phenotype. To investigate how the host genotype can determine microbial composition, the latter needs to be considered as the dependent variable. Low to moderate estimates of heritability (~0.05 to 0.40) for the abundance of different taxa in pigs have been reported by several authors (Camarinha-Silva *et al.*, 2017; Yang *et al.*, 2017) suggesting a partial genetic control of the microbial gut composition in swine. In an attempt of linking taxa abundance to host genomic variants (via GWAS), Cheng *et al.*, 2018 have identified two potential QTL regions on chromosomes 9 and 10.

Lu et al. (2018) modeled the  $\alpha$ -diversity of microbial composition over time obtaining heritability values for such measure between 0.1 and 0.4. Khanal et al. (under internal review) also estimated

X International Symposium of Mediterranean Pig 16-18 October 2019

Florence, Italy ABSTRACT

genetic correlations between  $\alpha$ -diversity and several carcass and meat quality traits, finding associations with growth and meat color traits.

The joint host-microbiome analysis can also be taken a step further by explicitly modeling genotype by microbiome interactions, thus eliciting potential differential impacts of microbiome compositions given different host genotypes. Recently, Khanal et al. (under internal review) found this interaction to be significant for meat marbling, firmness, and color.

Once the contribution of microbial composition to phenotypic variance has been obtained, economic selection indices could potentially account for such information. If the microbiome absorbs phenotypic variation, but the microbiome composition itself is not controlled by the host genome, then the microbiome is a purely environmental effect. In such case, if microbiome variability can be controlled (e.g. by manipulating diet, using pro/pre/anti-biotics), the heritability of the trait under selection could potentially be increased through the reduction in residual variance. Hence, better prediction of breeding values and faster genetic gain. If, conversely, the microbiome makeup is, to some extent, under direct control of the host genotype, then it could be considered as a correlated trait in selection. The genetic correlation between abundance of a given taxonomic unit or the  $\alpha$ -diversity and the breeding goal could be estimated and used in the selection index. This would be advantageous in cases where collection and sequencing of microbiome is cost-effective compared to direct phenotyping, which could be the case of meat quality or specific hormonal concentrations.

Lastly, gut microbiome composition could confer robustness and resilience to the animals. Individuals with a diverse, well-established microbiota should be more likely to tolerate environmental stressors, such as changes in diet composition, lack of essential vitamins in the diet, high pathogen exposure, etc. Since complex studies are required to understand such contribution of the microbiota to resilience, the swine breeders should carefully plan experiments and data collection protocols that include several time-points of sampling. This will allow to understand how microbiota composition can change over time and how this affects host's performance, resistance and tolerance.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

### **SESSION 2**

### ANIMAL BREEDING AND GENETICS

### ORAL COMMUNCATIONS

### Genomic analysis and selection signatures in local European pig breeds

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European local pig breeds are a major genetic resource and share relevant phenotypic characteristics, such as high adipogenic potential, rusticity and high meat quality. One key factor for the conservation, promotion and sustainable use of these breeds is their genetic characterization. In this study, we assessed genome-wide diversity and selection signatures employing SNP genotyping data from twenty European autochthonous pig breeds (Alentejana, Apulo-Calabrese, Basque, Bísara, Majorcan Black, Black Slavonian, Casertana, Cinta Senese, Gascon, Iberian, Krškopolje, Lithuanian indigenous wattle, Lithuanian White Old Type, Mora Romagnola, Moravka, Nero Siciliano, Sarda, Schwäbisch-Hällisches Schwein, Swallow-Bellied Mangalitsa and Turopolje) from nine countries (Croatia, France, Germany, Italy, Lithuania, Portugal, Serbia, Slovenia, Spain), as well as from wild pigs sampled in the Iberian Peninsula, and from commercial pigs (Duroc, Large White and Landrace) of Spanish and Italian origins. A total of 1150 DNA samples were genotyped with the GGP Porcine HD Genomic Profiler v1 chip, including 68,528 SNPs. Results pointed out some breeds with very low diversity for which conservation strategies should be applied. We searched across the genome for loci under diversifying selection based on FST outlier tests. Putative signals of selection were detected for regions containing genes involved in growth, muscle development, reproduction, metabolism, behavior and sensory perception, which may be considered candidate genes that may underlie differences in adaptation to specific environments and productive systems, and phenotypic traits. The findings provide relevant information for the implementation of further conservation and selection strategies.

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X International Symposium of Mediterranean Pig 16-18 October 2019

Florence, Italy ABSTRACT

## Genotype, gender and their interaction effects on piglet hypothalamic transcriptome

**ABSTRACT** 

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Swine production is mostly based on highly-efficient commercial breeds, but traditional local breeds (such as Iberian pig) are also used. Despite lower overall efficiency, recent studies have evidenced a major growth potential of Iberian piglets during early developmental stages, especially in females. The aim of this study was to determine the molecular events behind early-development in pure Iberian (IBxIB) and Iberian x Large White crossbreds (IBxLW) piglets without potential confounding effects of maternal environment, in order to explore possible interactions between genotype and sex on the hypothalamic transcriptome which could explain differences in growth potential. Hence, 16 sows were inseminated with heterospermic semen (mixed from Iberian and Large White boars) to obtain 44 purebred (IBxIB) and 28 crossbred (IBxLW) littermates. Piglets were weighed and measured periodically until 7 months old. At 2 months old, the hypothalamus of 10 IBxIB and 10 IBxLW animals were obtained for RNA extraction and the transcriptome analysis was performed by RNA-seq technology.

Crossbreds were heavier than pure piglets (females: P = 0.05, males: P < 0.0001). Differential expression analysis of transcriptome data conditional on genotype was performed separately for males and females, showing 198 differentially expressed (DE) genes in males, and 149 DE genes in females. The comparison between sexes showed 42 common DE genes. Only 15 out of these 42 common genes showed similar response (quantitative and qualitative) to genotype in both sexes. Potential interactions effects were observed, both qualitative (10 genes showing opposite genotype regulation in both sexes) and quantitative (170 genes with larger effect in males and 88 in females). The genes that showed qualitative interaction were related to the endocrine and nervous systems (TH, MPZ, PMP2), immune system (ISLR), growth (ISLR), growth (ISLR), and organismal development and growth (ISLR), ISLR), ISLR0, ISLR1. In summary, genotype\*sex interactions were found at the hypothalamus transcriptome level, that might explain the greater phenotypic differences observed between genotypes in males than in females.

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## Mining whole genome resequencing data from Italian pig breeds identifies selection signatures and putative causative mutations affecting phenotypic differences

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The genome of autochthonous breeds has been shaped by natural and artificial directional selection, constituting the main reservoir of animal genetic diversity. These genetic resources are adapted to different production systems and environmental conditions. In this study we mined variability in the genome of eight Italian pig breeds (three cosmopolitan breeds: Italian Large White, Italian Landrace and Italian Duroc; and five autochthonous breeds: Apulo-Calabrese, Casertana, Cinta Senese, Mora Romagnola and Nero Siciliano) and in Italian wild boars identifying selection signatures related to different morphological and production traits. Whole genome resequencing was carried out using paired-end Illumina sequencing from DNA pools (including equimolar DNA from 30-35 individuals each) obtained from all investigated breeds or populations (sequencing depth of about 40X). BWA-MEM mapped about 800 M read pairs on the Sscrofa11.1 genome version and about 20.5 M of variants were detected with CRISP coupled to an ad-hoc bioinformatic pipeline. About 15% of these mutations were not included in dbSNP yet and about 1% of all variants affected gene products. Fixation Index (F<sub>ST</sub>) and the Pooled Heterozygosity (H<sub>P</sub>) statistics were used to identify selection signatures on 100 kbp genome windows. A total of 40 genome regions (spread over 10 out of 18 porcine autosomes) included selection sweeps. Some of them, already reported by other studies, harbour genes affecting body shape/size (e.g. PLAG1 on SSC4, LCORL on chromosome 8 and CASP10 on chromosome 15) and coat colour (e.g. KIT on chromosome 8). Putative causative mutations were identified for all these regions. The results provided a first global catalogue of variability in the Italian Sus scrofa populations and identified chromosome regions and candidate mutations that might explain, at least in part, the phenotypic diversity of the investigated Italian pig breeds.

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ence, Italy ABSTRACT

## Influence of breed and dietary energy source on gut microbiota composition in Iberian and Duroc pigs

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Recent microbiome studies have led to new findings linking microbiota composition to multiple phenotypic traits. In the context of Iberian pig production, crossbreeding with Duroc and dietary fatty acid supplementation are two important factors influencing meat quality and overall animal performance. The study of the influence of breed and diet composition on microbiota is interesting, for the sake of understanding the relationship between the microbiome, the host metabolism and its phenotype, as well as for any potential use of the microbiome as a proxy trait for genomic selection. Forty-eight castrated male pure-breed pigs (29 Iberian and 19 Duroc) were distributed in two dietary groups, fed a control commercial diet with carbohydrates (CH) as energy source or a sunflower-enriched diet with high oleic acid content (HO). Animals were fed from 25 to 40 kg of live weight and slaughtered at the end of treatment. Stool samples were then collected from rectum for microbiome analysis. Illumina MiSeq paired-end sequencing was performed targeting 16S V3V4 amplicon. After quality control with Trimmomatic, 11.6M reads were processed following QIIME v1.9.1 open-reference OTU-picking approach, with SILVA-132 as reference database. An OTU abundance threshold of 0.005% was established for final quality-filtering. The final OTU table, including 1105 OTUs, was analysed with Phyloseq and Metagenomeseq R packages.

Results showed that richness, Chao1 and Inverse Simpson alpha-diversity indices were significantly higher in Iberian pigs (p $\approx$ 0.04,  $\alpha$ =0.05). Null hypothesis could not be rejected for Shannon index (p=0.073,  $\alpha$ =0.05). Bray-Curtis NMDS for beta-diversity showed a clear clustering by breeds, and PERMANOVA confirmed that breed and diet groups significantly differed in overall OTU composition (p=0.0002 for breed clustering and p=0.0119 for diet clustering corrected by breed,  $\alpha$ =0.05). Differential abundance analysis at OTU level was performed with the full dataset and within breed subsets. This led to the identification of 84, 43 and 30 differentially abundant OTUs between diets (q<0.1) in full, Iberian and Duroc datasets, respectively. These OTU belonged mainly to *Prevotellaceae*, *Ruminococcaceae* and *Lachnospiraceae* families.

In summary, our results showed that a sunflower-enriched diet is able to trigger differences on gut microbiota at the beta diversity level. Future research will target functional differences in these microbiota and their impact on the host phenotypes.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

Florence, Italy ABSTRACT

## Association study between SNPs in ACACA, ACLY, FASN, and SCD genes and fatty acid composition of backfat and muscle in Italian Large White pigs

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Over the last decades, the swine industry has prioritised lean mass growth, with a consistent decrease in the fat depots stored in carcass and muscle. In this scenario, the heavy pig industry has shaped distinct selection goals in Mediterranean countries such as Spain and Italy to develop selection schemes more suitable for the production of high-quality seasoned products. The amount and composition of backfat and intramuscular fat are important factors determining meat technological and nutritional quality, and therefore consumers' acceptance. The objective of the study was to test in Italian Large White pigs the associations of backfat and Semimembranosus muscle (SM) fatty acid composition (FAC) with Single Nucleotide Polymorphisms (SNPs) in the candidate genes Acetyl-CoA carboxylase alpha (ACACA NC 010454 g.38621011G>A), ATP citrate lyase (ACLY, two SNPs: NM 001105302 c.2956T>C and NM 001105302 c.3923T>C), Fatty acid synthase (FASN AY183428 c.265T>C), and Stearoyl-CoA desaturase (SCD AY487830 c.2228T>C). DNA was extracted from 536 SM samples; SNPs were analysed using PCR-RFLP and High-Resolution Melting analysis. The association study was performed with a mixed model in SAS software version 9.4. considering sex, slaughter day and genotypes as fixed effects, backfat thickness as covariate and the sire effect as random factor. After FDR-adjustment, associations with adjusted P-value<0.1 were considered significant. For backfat FAC the strongest associations were identified for the FASN SNP, with the T allele linked to lower backfat contents of stearic and arachidic acids, and increased amounts of dihomo-γ-linolenic and arachidonic acids. For SM FAC the most significant associations were found for FASN and SCD SNPs: FASN TT genotype was linked to higher muscle contents of myristic acid, while SCD TT genotype was associated with increased muscle contents of stearic and linoleic acids. Furthermore, an in silico analysis of the sequence flanking FASN SNP suggested that the T allele may disrupt a putative exonic splicing enhancer sequence therefore possibly affecting FASN transcription and protein activity. If the results will be further confirmed, the studied FASN and SCD SNPs could be of particular interest for better understanding gene interactions controlling backfat and muscle FAC in heavy-pigs and the two analysed SNPs may be promising markers for pig selection schemes aimed at improving FAC of meat products.

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## An online database for growth, carcass and meat quality traits for future breeding programs in local pig breeds

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Currently, breeding programs exist only for few local pig breeds in Europe. For some of them, no or very few phenotypic traits are recorded. To promote phenotyping, a dedicated database and a website were developed as part of the TREASURE H2020 project (https://treasure.ifip.asso.fr). Growth, carcass and meat quality traits as well as pedigree data can be recorded in a standardised way in order to characterise the breeds or to estimate genetic parameters of the traits (heritabilities and genetic correlations). This is the first step towards implementing breeding programs in European local pig breeds. Specifications were collectively defined to fit the needs of six local breeds: Basque (FR), Bísara (PT), Crna slavonska (HR), Gascon (FR), Krškopolje (SI) and Schwäbisch-Hällisches (DE). In total, 74 variables were identified representing animal herdbook information (e.g. animal identifiers, birthdates, pedigrees, sex), rearing and growth (e.g. breeding system description, weights and ultrasonic measurements on live animals), carcass (e.g. slaughterhouse classification data, carcass weights, ham and loin weights and traceability references, fat and muscle thickness) and meat quality (e.g. pH, colour, drip loss, intramuscular fat, tenderness, processing and slicing yields) attributes. Entry of the data can be done manually or through import programs. Format controls prevent errors. The database is compatible with the various identifiers (IDs) used in the different countries: animal IDs, breed, farm and slaughterhouse codifications. Great attention was paid to recording methods used for measuring traits. Thus, each carcass and meat quality phenotype are systematically and automatically associated with the method used on the slaughter place and date. This represents 35 additional variables. Method descriptions can be available on the website. The database is compatible with the main methods defined in the TREASURE meat quality evaluation toolbox. The website is already translated into several languages: English, Croatian, French, German, Portuguese and Slovenian, Other languages can be easily included. All stakeholders like breeding associations linked to local pig breeds (either those studied in TREASURE or other), are free to use these tools. Data can be hosted in a common database or in duplicate databases. They can be exported for further genetic analysis. Access to data recorded in the database should be submitted to the approval of the owners of these data.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

Application of DNA marker information to assure the integrity of autochthonous pig breed production chains

ABSTRACT

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Conservation programmes of livestock genetic resources should find sustainable economic solutions to support the cost of the needed in situ conservation actions. These animal resources are usually less productive than cosmopolitan breeds, therefore the development of mono-breed labelled products could contribute to assure profitability to the farmers. Through their link to a breed, the perceived quality of these products usually provides a higher market price than what is obtained by undifferentiated products. Based on these principles, several initiatives have been developed focusing on Italian autochthonous pig breeds. On the other hand, the market added value might represent a potential weakness of these pork chains due to potential high frequency of frauds derived by miss-labelling the breed of origin of the products. Therefore, the development of meat authentication methods can protect these chains from fraudsters. This work reports the application of DNA marker information to assure the integrity of Mora Romagnola and Cinta Senese pig breed production chains. The conservation programmes of these breeds are managed by the National Pig Breeders Association (ANAS). Mora Romagnola is mainly raised in the Emilia-Romagna region. Pigs have a typical black-brown coat colour. Cinta Senese breed is raised in Tuscany and pigs are characterized by black coat colour with a typical white belt. We genotyped almost all Mora Romagnola registered sows and boars (290 pigs) for polymorphisms in two genes (MC1R, affecting coat colour; NR6A1, affecting vertebral number). At the MC1R gene, all genotyped Mora Romagnola pigs carried alleles  $E^+$  (wild type) or e (recessive red) apart from five animals that carried the  $E^{D2}$  allele. At the NR6A1 gene, only two pigs carried the wild type allele. All pigs carrying the MCIR  $E^{D2}$  and the NR6A1 wild type alleles were eliminated from the Herd Book because their genetic profiles were not compatible with the breed specific genotypes at these two loci. A KIT gene polymorphism associated with the belted phenotype was genotyped in 150 Cinta Senese pigs. The belted allele was almost fixed in this breed and was not present in 650 pigs of other not belted breeds/populations. The KIT genotype was included as a Cinta Senese-specific feature defined in its Herd Book, Based on these results and actions at the Herd Book level, we designed two DNA methods to authenticate pork products that could be assigned to these breeds only if the analysed meat would have compatible genotypes at the considered markers. To our knowledge, these are the first examples in any animal genetic conservation programmes that established direct relationships between the breed Herd Books and DNA based methods to defend breed production chains from frauds.

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16-18 October 2019
ABSTRACT Florence, Italy

## The SUIS Project: actions for the conservation of Italian autochthonous pig breeds and for the improvement of sustainability.

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Since 2001, following the approval of the act DM n. 20871 - 6.3.2001 by the Italian Ministry of Agriculture, the Italian Pig Breeders Association (ANAS) has been working for a conservation of Italian autochthonous pig genetic resources. Its activities started with the collection and recording of pedigree information and the definition of breed standards, with distinctive phenotypic traits for each breed. Based on these actions and with the collaboration of local breeders, nowadays several autochthonous breeds are well established. The ANAS Herd Book lists seven traditional autochthonous breeds: Apulo-Calabrese, Casertana, Cinta Senese, Mora Romagnola, Nero Siciliano and Sarda. However, other actions to reach a sustainable conservation of these breeds are still needed. The SUIS Project, co-funded by the European Agricultural Fund for Rural Development of the European Union (http://www.anas.it/html/suis/homes.htm), has been established with these objectives. It involves several innovative actions which include the development of mono-breed product certification systems and the integration of genomic tools and information in the conservation program of these breeds, combined with a detailed phenotypic description of the different pig populations. Gene markers associated with breed-discriminant traits are analysed. For example, i) the KIT polymorphism associated with the belted phenotype is genotyped in Cinta Senese pigs ii) MCIR mutations, causing different coat colour phenotypes are genotyped in Mora Romagnola, Nero Siciliano and Apulo Calabrese pigs. A polymorphism in the NR6A1 gene, associated with teat and vertebral numbers and that can be used to distinguish wild boars from domestic pigs, is analysed in Mora Romagnola pigs. The RYR1 gene polymorphism causing the pale, soft and exudative (PSE) meat defect is genotyped in Apulo-Calabrese and Nero Siciliano pigs. All animals without any breed specific genotypes have been then eliminated from the Herd Book. The SUIS Project, through the integrated use of phenotypic and genomic information and data, supported the development of high quality local pork products and breed specific production chains by eliminating deleterious alleles and creating a direct genetic link with the Herd Book of these breeds.

### Acknowledgements

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38

## **Exploring genetic diversity in Nero di Parma pig breed to develop** strategies for conservation and management

ABSTRACT

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The Nero di Parma is an Italian native swine breed, officially approved in 2006 as a hybrid breeding pig and recognised in the 2016 as a breed, which has steadily gained popularity in the Italian territory. Already in the nineties, several policies were implemented to conserve this breed as representative of a traditionally reared breed in the North of Italy before the introduction of commercial breeds. A pedigree analysis was carried to evaluate population structure and investigate the current breed's genetic variability. The pedigree data contained 14,485 animals which was used to estimate population parameters in Endog 4.8. Demographic and genetic parameters were estimated on the whole population and on selected subpopulations to assess potential genetic diversity in breeding animals (n. 978), in alive animals (n. 1,403) and in alive and breeding animals (n. 336). The mean complete generations equivalent was equal to 6.47 in the whole population and it reached a mean value of 7.94 in the alive animals indicating a remarkable pedigree depth. The effective population size was equal to 129 individuals in the whole population, while it decreased to only 11 animals in the alive population. Two ancestors explained 50% of the observed genetic variability and 99.8% of the population showed a certain degree of inbreeding. Average inbreeding was 0.28 in the whole population, whereas it reached 0.31 in the alive animals. Nevertheless, the average inbreeding of alive sows and boars used for breeding was equal to 0.27, which highlights the current support service provided by the breeding association aiming to use less inbred animals in the breeding program. Throughout the studied period, inbreeding steadily increased, reaching in the last birth year cohort a mean value equal to 0.32. The rate of inbreeding for the last generation, considering a mean generation interval of 2.46 years, was equal to 6.84%, which vastly exceeds what FAO advices to preserve a breed in the long term. Our study unravelled the state of genetic diversity in the Nero di Parma breed, highlighting that further breeding strategies to optimise the contribution of breeding animals in the coming generations are needed to ensure long-term survival of this breed.

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Florence, Italy **ABSTRACT** 

### Genetic evaluation of the longevity of the traditional Italian Large White and Italian Landrace breeds

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Longevity can be considered as an indicator of the sow efficiency, both biological and productive, and of its welfare status. Longevity is a complex trait influenced by genetic and environmental factors which can only be detected after the reform or death of the sow. This trait can be measured in different ways, such as the duration in days of her productive life or the number of farrowings the sow's career, etc.

The objective of this work was to illustrate a simple but effective genetic index developed by Italian Pig Breeders Association (ANAS) for sow longevity using a BLUP Animal Model Single Trait.

Observations considered by the Index were the number of farrowings in each sow career. Only data from culled sows were considered, and sows with more than 12 farrowings were censored to 12. Heritability of longevity was estimated for the Italian Large White  $(h^2 = 0.14)$  and Italian Landrace  $(h^2 = 0.11)$  breeds using the data available in the ANAS database. Males and females were selected based on their pedigree index for longevity.

The selection for longevity is performed together with selection on traits important for meat quality for seasoning, such as maintaining subcutaneous fat coverage and weight losses of hams in brine. Productive traits such as Growth rate and Feed Conversion Index are selected for only insofar they do not hamper meat quality traits. This is because the final destination of ANAS pigs are the PDO productions (Parma and San Daniele hams and others).

Correlations among Longevity Index and the Total Merit Index were r=0.54 and r=0.47 for Italian Large White and Italian Landrace, respectively. This shows that the Italian selection for seasoning quality of meat is compatible with sow resilience and sustainable from an ethical point of view.

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### Genetic programs for the preservation of Italian autochthonous pig breeds: results and future prospects

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The aims of the breeding programs for the Italian autochthonous pig breeds are maintain the genetic variability, reduce inbreeding, improve phenotypic and genetic characterization, promote sustainable pork chains. In the 1997 ANAS set up the first program for Cinta senese and from 2001 the programs for the other local breeds. There are rules to maintain a high rate between boars and sows, to assure a reliable individual identification of the animals and of their pedigree information. The breeders can use information registered in ANAS data Bank and available on web site to simulate mating choose and others. Pedigree information stored on ANAS databank have been analyzed with the software ENDOG (Gutierrez and Goyache, 2005) developed for the analysis of small populations. Some population parameters are estimated: number of equivalent generations, pedigree completeness, reference population, number of founders, effective number of founders and ancestors, Moreover trend of the average inbreeding and kinship are estimated. Analyzing this trend emerged that average kinship value is higher than the average inbreeding coefficient in Cinta senese (F=17.42%, AR=23.03%) and Mora romagnola (F=19.66%, AR=25.21%) breeds where the exchange of boars is a traditional and usual practice. In local breeds where this occurrence has been less intense, the average kinship between subjects present in different farms is limited, while inbreeding is higher (e.g. Apulo calabrese breed F=14.41%, AR=10.10%). The average inbreeding values recorded in the last years in ANAS data Bank show a quite stable level in all local breeds. These results confirm the efficacy of the breeding programs to preserve the Italian pig biodiversity. In the future some genomic information can be used to improve characterization of each breed and for the breed authentication of the products. This is an important issue to contrast the frauds and improve the value of special pork market niche.

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### Pig farming in the Mediterranean area: social, environmental and economic aspects

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Many studies date the pig domestication 9000 years ago in the Near East. More recently zooarcheological studies have shown that another parallel domestication occurred in the Nera East. Since than pig has been a part of the culinary history of humanity. Nowadays pork is the most eaten meat in the world (15.8 kg/capita/year) followed by poultry (13.6 kg/capita/year), beef (9.6 kg/ capita/year) and finally sheep and goat meat (1.9 kg/capita/year) (FAOSTAT, 2014). The 40% of all meat consumed globally is pork, overcoming chicken (29%) and beef (24%). Since the 1970s the world's pork industry has been growing up, reaching in the 2018 a global production of more than 113 million tons. Worldwide more than 769 million of pigs are reared. In 2018, China accounts for almost a half of the world pork production, with about 55 million tons of pork produced annually. Nevertheless, China is a net importer of pork meat being the first consumer of pork meat in the world. The EU countries are together the second consumer and the second largest pigs' producer in the world with about 24 million tons per year. German and Spain are the leading country at EU level. The USA, the third largest in numbers of pigs, has the 10% of world pigs. The EU, USA and Canada are in prominent position as pork meat exporter countries providing the 90% of China's pig meat imports. Denmark, Spain and German are the leader countries for annual per capita consumption of pork meat. The world consumption of pork is showing a general stability in the last years with some exceptions. Since 2015 increases are observed in Japan, Russia, UK, Czechia and Spain, while consumption declines are accounted for China, Italy and Germany. Different reasons can explain such situations; for China ASF occurrence and prices increases may have lessened pork demand, while in the case of Italy and Germany, awareness about animal welfare, health and especially for Germany the effects of Muslin population habits may have played a role in reducing pork consumption (Deblitz 2018). The 78% of pork meat is consumed as processed food, including ham (31%), sausage (19.8%), bacon (2%). Consumers are very sensitive to the convenience and easy preparation of meat, European consumers rank convenience as the second attribute in selecting transformed pork (Resano et al., 2011) while it is third for importance in the case of fresh pork meat. Consequently, technology innovation efforts have focused on convenience aspects to maximise customer satisfactions (Troy & Kerry, 2010). Consumers' satisfaction is highly influenced also by price, especially in purchasing fresh meat, while in the case of processed pork and pork meat products the price has a minor role in consumer choices, since this products are perceived as high quality, differentiated and branded products in which quality attributes are determinant in purchasing decisions. Since fresh pork meat demand is very influenced by price and pork industry is affected by economy of scale, swine farms be likely to be larger and highly specialised hosting just a phase of the production cycle and the farms raring the pig from the breeding to the slaughter are limited in number. The supply chain of pork meat is very intensive and awareness on its effects in terms of sustainability, animal welfare and meat quality are raising concerns among consumers (Verbeke 20101). Recent study shows that Danish consumers have negative perceptions of pork and poultry meat in terms of animal welfare while referring to lamb and beef they do not have such a negative perception (Holm 2010). In general pork meat is considered fatty and less healthy in comparison with beef and generally lean pork is preferred to fatty pork except for Japan and South Korea areas where consumers prefer fatty pork (Verbeke 1999). Meat is central element in the diet, but meat consumption pattern is changing rapidly.

SOCIO ECONOMICS

**SESSION 3** 

MAIN LECTURE

X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

**ABSTRACT** 

ABSTRACT

The consumption pattern of meat is rapidly changing. There is evidence that the consumption pattern of pork meat is highly differentiated among and between countries in function of cultural, social, economic and ethical issues. Worldwide the annual per capita consumption of pork meat varies from more than 50 Kg per capita in German, Denmark and Austria to 3 kg per capita of south Africa to be almost absent in Muslim countries. According to Holm (2010) in Copenhagen social condition influences consumers habits towards pork fresh meat. Middle class people consider fresh pork meat as inferior meat and they do not serve pork meat as formal meals, while workers class don does not have these preconceived views and consume fresh pork frequently. Lamb is considered more modern, healthy and middle-class consumers show more willingness toward this meat. Recent study has revealed that European consumer preferences toward processed and pork meat products are higher if they are made by mixed meat (poultry or lamb meat) and without chemicals. In the future, the pork meat sector will face an increasing competition on prices for not differentiated and unbranded products and an increased demand for sustainable healthy, ethical and convenience product attributes. In this perspective product differentiation and innovation together with technology innovation will play an important role in enhancing sector competitiveness and sustainability.

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### **SESSION 3**

### SOCIO ECONOMICS

### ORAL COMMUNICATIONS

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ABSTRACT

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## Consumer acceptance and market potential of products from Krškopolje pig

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Preservation of agricultural genetic resources and sustainable primary production are priority areas of agricultural research and innovation policies. There is an increased consumer interest for traditional resources and products coming from less-intensive rearing, with more care for animal welfare, which could be an asset for the untapped and endangered local pig breeds and their enhanced use. In the frame of H2020 project TREASURE, we studied market potential for the products of the only Slovenian autochthonous pig breed, Krškopolje pig. To evaluate consumer acceptance for the products of Krškopolje pig, 131 consumers, all of them eaters and purchasers of pork or pork products, were recruited and asked to evaluate 4 products/salami; low price commercial salami, high price (premium) commercial salami, traditional salami from Krškopolje pigs, traditional salami from Krškopolje pigs with innovation (without nitrites). The assessment consisted of three steps; in the first the consumers received the products without any information (blind liking), in the second round the consumers were asked to provide the assessment based on product description (expected liking), and in the third round the consumers received both, the products together with the description (informed liking). Consumers were asked to evaluate the acceptability using a 9-points Likert type scale (from "dislike extremely" to "like extremely"). Market potential was evaluated using "World café" technique in focus group discussion with stakeholders (n=12). SWOT (strengths, weaknesses, opportunities and threats) and 4P marketing strategies (Product, price, promotion, place) analyses were conducted. With regard to product liking tests, there were no differences between different salami observed in the blind and in the informed test (idem no difference between blind and informed liking within product), whereas the differences were noted for the expected liking, with the highest expectations for the salami from Krškopolje pig (in particular the one without additives-nitrites). The results of this study show that the expectations of consumers were high, but not met; i.e. were disconfirmed. The reason may be in too high expectations and/or unfamiliarity of consumers with such products. Still, high consumer expectations for the products from Krškopolje pig are a good signal for the building up of the value chain based on this breed. In a workshop, the stakeholders identified the pros and cons related to the products from Krškopolje pig and the building up of value chain. The recognised assets were mainly non-intensive traditional rearing, good quality and taste, and cultural heritage, and the main shortcoming was unorganised value chain together with lack of product standardisation. Stakeholders have also identified that the products of this breed are interesting for the higher priced niche supply of both, fresh and dry-cured products, for the innovative culinary offer in best restaurants. Direct sales and business or protocol gifts were also identified as important marketing strategy. Likewise the importance of branding strategy (trademark) was emphasized.

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## Analysing producer opinions about traditional pig keeping in Hungary

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Hungarian traditional pork consumption has been limited as a result of relations established between fat consumption and African swine fever (ASF). The objective of this study was to determine the attitudes of Hungarian farmers to traditional pig keeping. For the analysis, in-depth interviews have been carried out with experts, and producers of the mangalica product chain. The research was based on a non-representative sample; the selection of interview subjects was carried out by means of purposive sampling amongst the producers, with the aim of involving small, medium and large farms in the sample.

In the course of our study it was found that the whole mangalica segment is three-polar on the level of every product path actor. These three poles can be classified to the levels of small, medium and large producers, processors and dealers.

Three levels of the producer side: The high number of small enterprises – in the case of the mangalica breed the ones with less than 30 sows - represents only a small share within the total stock. These producers play a major social role as a result of bio-farming and rural tourism, which might represent a significant potential for the Hungarian economy. Medium enterprises owning 30-100 sows are able to produce for market sales besides their own use, and they also play an important role in gene preserving. Approximately one-third of the Hungarian mangalica production units belong to this category and they own almost one-fourth of the total sow stock. The relatively large producers own 80% of the porkers; they mainly produce for food chains or foreign markets in large quantity and standard quality. In order for small, medium and large enterprises to be able to cooperate effectively, these three production unit types have to cooperate with each other. Small and medium enterprises mainly breed/fatten clean blood mangalica. With their extensive or semiextensive breeding, they are unable to supply foreign markets either in quantity or in proper quality. However, in large enterprises, the crossbred mangalica (Mangalica x Duroc) especially serves foreign demands with proper quantity and always standard quality. The Mangalica-Duroc crossbred type is accepted in both domestic and foreign markets, as a result of its many advantageous properties. Besides the more favourable fertility and growth vigour, the processors need the hybrid mangalica, because slaughterhouse technology would not be able to process the high amount of fat and certain quality can be produced in better quality from crossbred animals.

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ABSTRACT

## Experiencing new ways of designing pig disease management programs: emergence of a form of "middle management" of Aujeszky disease in Corsica

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In France, for infectious disease of major importance, such as Aujeszky disease, the management strategy design and implementation fall under the responsibility of animal health authorities, following a vertical and hierarchical organization involving veterinarian and specific farmers' organizations (GDS). In Corsica, such organization has been challenged for 40 years by Aujeszky disease: whereas continental France has been free of the disease since 2008, the successive plans to eradicate Aujeszky disease in Corsica have failed despite the efficacy of the vaccine available, and the situation appears to be in a dead end.

In 2016 researchers from INRA and Regional farmers association (FRGDS) initiated a cycle of participative workshops to understand the reasons of failure of classical management approaches, and to build a new strategy. Involving a large diversity of actors (farmers, hunters, veterinarians, state authorities, researchers,...), these workshops allowed to collectively reformulate the terms of the Aujeszky disease management situation in Corsica and to propose an original form of organization to deal with it. This communication proposes to shed light on two main results: i) the collective process of rationalization of management measures, taking into account the specificities of the Corsican pig sector, and ii) the output of the process (the strategy), that produces a new form of organization, legitimating new actors in decision making process and new forms of management actions and collective procedures.

Following a classical perspective in management & organization sciences developed by scholars who bridged *sensemaking* and *organizing* activities, we explain how management measures get progressively rationalized regarding geographical, temporal and sociotechnical scales that "make sense" for actors, and that allow to take into account the diversity of Corsican pig farming systems. At the difference of classical management strategies, the strategy proposed by the group relies on a micro-regional approach, a step by step process, and new capabilities and roles for certain stakeholders, especially farmers. Thus, by taking the problem through a bottom-up approach, involving stakeholders, our approach proposes a perspective inversion to deal with such diseases, especially in territories characterized by semi-extensive and diversified pig farming systems, which does not "fit" in classical disease management approaches.

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### A qualitative and participative approach to analyse different strategies of valorization of Noir de Bigorre pork chain

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The Noir de Bigorre (NDB) French regional pork chain produces fresh pork and dry cured ham (under Protected Designation Origin PDO label) from Gascon purebred pigs. Within TREASURE project, a focus group was carried out with stakeholders of the NDB chain to better know and collect their perception of the way its products are valued. The objectives of this participative approach were to conduct a Strength, Weakness, Opportunity and Threat (SWOT) analysis of the NDB chain and products, as well as to assess the feasibility of potential marketing strategies for creating added value and matching market demands. A World Café (WC) was conducted with 11 representative stakeholders of the supply chain (farmers, processors, retailers, consumer, governance...). Participants were allocated to four tables. The first WC session was focused on the SWOT analysis and the second session on a 4P (price, products, place, promotion) approach. Then main ideas of the discussion on 4P were selected after voting to conduct an Analytical Hierarchical Process (AHP). Lot of strengths and opportunities have been highlighted. PDO label and high sensorial quality of the products enhance attractiveness and values of the NDB chain. NDB chain characteristics (farming system, naturalness, history, heritage and culture) meet social expectations of consumers. Improvements points have been underlined through weakness and threat regarding sanitary risks and data management. AHP method allows the classified relative importance of each item/strategy within and between the 4P topics. We observed a strong proximity between the strategies preferred by stakeholders. Potential evolutions of the carcass payment system for farmers and the information provided on loin and ham were mentioned. The development of the coverage of local stores in PDO area was a potential strategy suggested to improve local visibility of the products. Promotion of the NDB products is mainly linked to its story. To support products from the animal to tasting is important and needed. This participative approach gives elements to guide the reflections of the NDB chain actors with a constructive and optimistic view to the future.

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ABSTRACT

## An insight about the Spanish quality standards for Iberian pig products by means of stakeholders in-depth interviews

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The Iberian pig sector is of great economic importance within livestock production in some Spanish regions such as Extremadura, Andalusia and Castilla and León. These regions include a large number of pig farms with different characteristics. As well as, there is a developed industrial sector which transforms the Iberian products through different capacity and commercially-oriented factories. Since 2014, the Quality Standard (RD 4/2014 of January 10) for meat, ham, shoulder and cured loin from Iberian pigs controls all the production processes of both raw material and transformation processes of the product. All producers (processors and farmers) have to comply with the aforementioned standard to be able to market the products under the name of "Iberian". Since the Quality Standard was introduced, different stakeholders (farmers, meat processors managers, certification bodies, policy makers, consumers, etc.) had to make a huge effort to adapt the production and processing systems to succeed in the new market conditions.

At present, some years after the Quality Standard enforced, some of these stakeholders are demanding amendments to this regulation. These changes are going in different directions according to the collective that they represent. For this reason, a qualitative research has been developed with the objective of studying the perception of farmers and meat processors of the Iberian pig sector on the requirements of the Quality Standard that is currently in force, as well as the conditions in which it is being enforced. To this end, 14 in-depth interviews were carried out with farmers and producers of Iberian products of different characteristics during the period from October to December 2018.

The results show that there are a number of aspects of the Quality Standard regulations in which consensus has been reached among most of the participants, in such a way that it seems appropriate to keep them under current conditions in the case of a possible regulatory modification such as: the minimum weights of slaughter and minimum weights of replacement in each system, the denominations of sale and labeling system of the products.

There is also some consensus regarding the benefits that the implementation of the Quality Standard has meant for the sector, while some points have been identified as potentially improvable like, the system of inspection and certification.

On the other hand, there are differences in aspects related to the compulsory minimum ages of slaughter of the animals in each feeding and husbandry conditions (Acorn-fed, Fattening on farm or Indoor Fattening). Finally, processors and farmers are more oriented to the production of Iberian pigs and products not linked to the dehesa ecosystem (green and white labels) are more prone to regulatory changes oriented to reduce of the minimum age of slaughter of pigs.

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**SESSION 4** 

FARMING SYSTEM + ANIMAL HEALTH

MAIN LECTURE

Italy ABSTRACT

### Iberian pigs and dehesa: an agroforestry system

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The dehesa is the most unique and representative agroecosystem in the southwest of the Iberian Peninsula (>4 million ha). It is an area with trees that are more or less dispersed and a well-developed herbaceous stratum, the shrubs having been mostly eliminated; It has an agricultural (ploughed land in long term rotation) and stockbreeding origin; and its main use is for extensive livestock, using grasses, fodder and tree fruit. Hence, in the dehesa, trees are key to feeding livestock and animal grazing is an integral management component. Palynological analysis of Neolitic sites have shown evidence of its existence for 6000 years, when the Mediterranean forest was cleared to have grasslands while conserving and selecting the *Quercus* trees to produce fruit (bigger and sweeter acorns); *Q. ilex rotundifolia* being the priority species.

This system is highly appreciated by society because it is rich in biodiversity, an important carbon sink, a tool for fire prevention, a traditional culture, an appreciated landscape and it supports rural development. It has been consequentially qualified as "natural habitat" to be preserved, within the European Union Habitats Directive, being the largest high nature-value system in Europe. However, most of these values do not produce any kind of benefit to farmers and they do not receive any kind of state support from these contributions. This is both a resilient and a fragile system; its resilience derives from the perseverance of its farmers, and its fragility is its susceptibility to unfavourable economic factors that influence its profitability and, hence, all the necessary activities for its maintenance. The traditional dehesa livestock were indigenous breeds (mainly sheep from autumn to spring and then pigs during autumn and winter) with lowstocking densities (0.5-1 suckling ewe equivalent/ha), extensive tillage alternating with 3-20 years of fallow (to prevent shrub invasion and to supply fodder) and numerous marginal uses. The ideal denseness of O. Ilex is 20-50 adult trees/ha; and its sustainable stocking rate suggested is 0.2 - 0.4 LU/ha (Olea and San Miguel-Ayanz, 2006). Hence, cattle participate in the dehesa creation and are indispensable to its maintenance, while silviculture and agriculture are secondary once a dehesa is kept in equilibrium with grazing. Due to this diversification and efficiency, the dehesa was also a very versatile system and was able to successfully satisfy human requirements, which has been the secret of its survival (Olea and San Miguel-Avanz, 2006), However, nowadays, mixed livestock of cattle, sheep, pigs. etc. accounts for its largest fraction of income; although a key species, such as sheep, has unfortunately kept the same market value over the past 30 years. Conversely, the Iberian pig is the most appreciated priced livestock, because of the outstanding quality of its cured products.

The productivity of acorns is 10 times higher in a managed dehesa compared to a dense Mediterranean forest. The mean acorn yield is 300 to 700 kg/ha, with yields of 8-14 kg/tree for *Q. ilex* (Rodríguez-Estévez *et al.*, 2007). The average weight of an acorn is 5.7 g, with means of 4.4 g and 2.5 g of kernel fresh and dry matter (DM), respectively (Rodríguez-Estévez *et al.*, 2009). Shell composition has a very high level of tannins and lignin, which affects its digestibility, although the Iberian pig breed is capable of peeling acorns; while kernel has a very high level of glucids (80% of DM) and lipids (5-10% of DM), with oleic acid content upper 60%; however, the protein level is very low (4-6% of DM) (Rodríguez-Estévez *et al.*, 2008). On the other hand, the autumn production of grass has been estimated at 200–500 kg DM/ha.

The whole traditional productive cycle of Iberian pigs was organized to make them capable of foraging acorns during their finishing phase (montanera, meaning pannage). An important aspect of

X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

### **SESSION 4**

FARMING SYSTEM + ANIMAL HEALTH

ORAL COMMUNICATIONS

## Quality of dry-cured ham from Krškopolje pig – comparisom of organic and conventional housing system

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Data on the only Slovenian autochthonous breed, the Krškopolje pig (KK), are quite scarce, including the information on the effect of rearing system, aptitude for processing or product quality. In the present study, dry-cured hams were produced from KK fed equivalent diets and reared under conventional (CON, indoor housing) or organic conditions (ECO, access to outdoor area, alfalfa hay supplementation). As the high incidence of RYR1 gene mutation is known for KK, its effect on drycured ham quality was also tested. Prior to processing, green ham traits (weight, pH, colour - CIE L\*a\*b\*) were measured and processing loss recorded. At the end of processing (515 days) colour, chemical composition (moisture, proteins, lipids, proteolysis index) and rheological traits were determined. The data were analysed by SAS statistical software and GLM procedure, testing the effect of rearing and RYR1 in the model. The interaction of the effects was also tested, but proved insignificant (P>0.10). Regarding the green ham traits, ECO pigs had lower gluteus medius muscle (GM) pH (P<0.05), and lighter (P<0.10), more red (P<0.05) and more yellow (P<0.05) colour of GM and gluteus profundus muscle than CON, Compared to CON, dry-cured hams from ECO group had lighter semitendinosus muscle (P<0.10) along with higher biceps femoris muscle (BF) cohesiveness (P<0.05), springiness (P<0.05) and chewiness (P<0.05) and lower semimembranosus muscle force decay coefficient (P<0.10). As for the RYR1 effect, the mutated allele was associated to lighter colour of green (GM, P<0.05) and dry-cured ham (BF, P<0.10), higher weight loss during salting phase (P<0.05) in addition to lower protein and higher lipid content in BF (P<0.5).

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## Evaluation of muscle and fat deposition in three Iberian pig lines in two feeding systems by serial ultrasound scanning

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The objective of this study is to compare the growth of fat and meat among three genetic lines of Iberian pigs in two feeding systems. Castrate male Iberian pigs (n=60) belonging to three genotypes were used: 20 Lampiño (L), 20 Torbiscal (T) and 20 Retinto (R). At an average body weight (BW) of 90 kg, half of the animals (10 of each line) were confined in large pens and finished in semiintensive conditions using concentrate, the remaining 30 animals were fed in extensive conditions with acorns. Pigs were weighed and scanned with ultrasound 4 times during the finishing period. The loin (at the last rib level) and the gluteal area were scanned. At the loin level, internal, middle, outer and total subcutaneous fat layers, and loin area (ISF, MSF, OSF, TSF, LA, respectively) were measured. Gluteal depth (GD) and gluteal subcutaneous fat (GSF) were also measured. Measurements were predicted at 100, 120, 140, 160 and 180 kg of BW by individual regressions. Comparing L vs R genotypes, when fed on acorns, ISF, MSF and TSF layers grew faster in L than in R genotypes, but OSF and GSF growth did not differ among lines. When fed with concentrates, however, ISF, MSF and TSF grew equally in L and R, but OSF and GSF growth differed among lines. As for muscularity, when fed with acorns, LA grew more in L than in R genotypes and LA and GD grew similarly in both genotypes, but there were no differences among lines in GD growth when fed with concentrate. Comparing R vs T genotypes, when fed on acorns, MSF and ISF grew faster in R, but OSF, TSF and GSF grew faster in T. However, when fed with concentrate, ISF, OSF and TSF grew more in T, while MSF and GSF grew faster in R. As for muscular deposition, LA grew faster in T genotype and GD grew faster in R genotype in both feeding systems. In conclusion, tissues grew differently depending on genotype and feeding system. With a similar weight of 160 kg for all lines, Lampiño was more lipogenic in the acorn system, with a muscular loin deposition similar to that of the other genotypes. In addition, when fed with concentrate, Lampiño genotype deposited more loin muscle than the other two genotypes. On the other hand, the Torbiscal genotype deposited less subcutaneous fat and more loin area than the Retinto in the acorn system, but Retinto can produce hams with more muscle and less fat but thinner loins than the Torbiscal. At 180 kg, Torbiscal pigs fed with concentrate can produce the thickest loins but with the smallest subcutaneous fat thickness.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

## Effects of selenium-biofortified triticale feedstuff on meat quality and antioxidant capacity in extensively raised Iberian pigs

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Extensive Iberian pig production involves high costs that can be compensated by exporting high quality products. However, due to its valuable high intramuscular fat content and lipid composition, Iberian pork is very prone to rancidity, especially certain highly infiltrated prime cuts. Selenium (Se) supplementation delays meat post-mortem oxidation reactions. Organic Se (e.g., selenomethionine) reaches tissues more efficiently and durably than the inorganic forms, which may even act as a toxic prooxidant at high concentrations. Feed supplementation with organic Se is expensive unless it is done through agronomical biofortification (incorporating Se to crops). This biofortification is effective and safe for producing animal feed and human food. This study aims to:

1) Determine the effectiveness of Se-biofortification in triticale crops. 2) Evaluate the use of Se-biofortified triticale grain in Iberian pigs during the pre-finishing vs the pre-finishing + finishing periods to determine the permanence of Se and the effects on pork antioxidant capacity.

A 4-ha triticale plot was sprayed with sodium selenate (10 g Se/ha) at stage 30 of Zadoks scale (beginning of stem elongation). Grain yield was 1535 kg/ha, with a concentration of 430±22 μg Se/kg, which was greater than in control plot (<125μg). Starting at 7 months of age (m), castrated Iberian pigs were fed with standard concentrate containing 45% of regular triticale (Control; Ctrl, n=10) or the Se-biofortified triticale up to 12 m (Se-1; n=9) or up to slaughter time at 14 m (60 days more; Se-2; n=10). Blood samples were collected at these 3 time points for Se content and metabolic parameters. Pre-slaughtering, loin and gluteal echography was performed. Carcass and prime cuts were morphometrically analyzed, and meat samples stored for Se content and meat quality analyses (before and after 7-day maturation; d0, d7).

Meat Se content and blood analyses are not yet available. Treatment did not affect BW. In both treated groups (Se-1, Se-2), loin pH at d0 was higher (6.06 vs 5.63), "L" and "b" colors at d7 were lower, and cooking loses were lower. Loin TBARS was greater at d7 in Ctrl samples (more oxidized after maturation). Regarding loin texture at 20% compression, adhesiveness, springiness and resilience were greater for Se-2, whereas, at 80% compression, hardness was greater in Ctrl vs Se-treated groups and cohesiveness was greater in Se-2 vs Ctrl. In conclusion, these preliminary results suggest (for the first time, as far as we know) a great potential for this sustainable strategy to increase meat antioxidant capacity through agronomical selenium biofortification for animal feeding and for Iberian pigs in particular, even after a 2-month period of concentrate withdrawal, which is required for montanera-finished animals.

### Acknowledgements

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ABSTRACT

## Slaughterhouses and pig farmers in Corsica: a systemic and geographical approach of a sanitary surveillance system

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Nowadays, slaughterhouses have become a standardised tool being fully part of the industrial food chain process. In France, they also constitute one of the sanitary control system's main bottlenecks where all animals meant to be transformed in meat sold for human consumption must be subjected to *ante* and *post-mortem* inspections. In Corsica, it was approximatively estimated that 50% of the pigs are slaughtered in slaughterhouses. As a standardised mandatory tools, they are relatively new and they face an heterogeneity of farming systems where pigs' breeders are mainly "processorsfarmers". It raises questions about their sanitary surveillance role: to what extend is it used by breeders; does it grasp the animals' sanitary situations; does it fit with the region's farming systems diversity? In order to answer those questions, the paper is based on a mixed methodology. First, data from slaughterhouses are analysed in order to grasp the zootechnical, social and spatial diversity of their use. Second, semi-structured interviews with breeders are mobilised in order to develop a comprehensive understanding of the relationship between farmers and slaughterhouses.

The first part of the paper shows that pigs' farmers using slaughterhouses are spatially well distributed all over the main pigs' areas. However, quantitative data shows a great diversity in the number of slaughtered pigs by farmers (1 to more than 200 per year) and in pig characteristics (breed; age; weight from 35kg to 240kg). This result pledges for two hypotheses. First, there is still an important account of small familial pigs' breeding in Corsica. Second, many farmers slaughter a part of their pigs at home. The paper shows that both hypothesis are accurate.

The second part of the paper focusses on breeder's perceptions on slaughterhouses in order to explore their reasons for not using it. Not using slaughterhouses depends on several factors: commercial outlet (being or not being commercialised in official restaurants or stores); journey to slaughterhouses (travel time, road types etc.); the number of pigs to slaughter (farming systems) or bad previous experiences with slaughterhouses (eg. receiving bad quality carcasses). In conclusion, for the slaughterhouse to be an efficient sanitary surveillance tool, the specific relation to the slaughterhouses due to the fact that breeders are also processors of pork products, is strongly needed to be taken into account.

### Acknowledgement

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## Using local knowledge to characterize Corsican pig farms practices for an epidemiological purpose

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X International Symposium of Mediterranean Pig

16-18 October 2019

Florence, Italy

In Corsica, the pig sector is characterized by a large diversity of farming systems, mainly based on traditional and extensive practices. Although this sector configuration promotes the various agro-ecosystems of the island and adds value to agricultural products, it also facilitates direct and indirect contacts between pigs and wild boars, which are suspected to favour transmission of infectious diseases between suids and to humans. In order to improve our knowledge and to model pig/wild boar interactions, we need to collect accurate data on land use, spatial distribution of herds and farming practices. However, this is a major issue in Corsica because such data does not exist in official census and databases. Therefore, we propose a two-step methodology to collect, gather and analyse these data by mixing methods inspired by social sciences and epidemiological approaches. First, we established a method to collect pig farming data in several micro-regions. From different criteria like the strong inter-knowledge within the Corsican population and because of the complexity and cost of field work due to the topography of the island, we implemented a key-informants based method. This questionnaire approach using local knowledge allowed us to gather regional information on formal and informal pig production figures, map the pig density and extract pig farming practices facilitating interactions with wild boars. Subsequently, using a MCA (multiple correspondence analysis) analysis, we established different clusters of practices describing different categories of farming systems, which we then submitted to a group of experts, composed by technicians and breeders. Using participatory methods with this group (focus group discussion, ranking, proportional piling), we obtained a quantitative graduation of the risk for each farming system. Finally, by mapping our results in GIS layers, we show evidence that the risk of interaction with wild boars is strongly heterogeneous in the territory and can be influenced by the pig sector configuration and practices. Thus, our study gives an example of the potentialities of local knowledge and participatory methods to better understand ecoepidemiological issues, and to highlight the existing gap between official databases and the reality of pig farming production distribution in the Corsican territory.

### Acknowledgments

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

### **SESSION 5**

### ANIMAL BREEDING AND GENETICS

### ORAL COMMUNICATIONS

## Association analyses for intramuscular fat content on purebred Iberian pigs using a costumed SNP panel from RNAseq data

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The high intramuscular fat content (IMF) of Iberian pigs confers them a high quality of meat and dry-cured products. This trait is suitable to be included in breeding schemes and to be selected using marker assisted selection (MAS). One approach for detecting potential candidate genes and markers is RNAseq, through the alignment of sequencing reads from the transcriptome to a reference sequence. In the current study, we identify polymorphisms in differentially expressed genes and lncRNAs in Longissimus dorsi (LD) muscle of Iberian pigs divergent for IMF breeding value, and functionally related with lipid metabolism and muscular growth that were detected in a previous work. The aligned Illumina reads from LD RNA samples of six animals of each divergent group were used in the SNP calling. Genotypes were called for each individual sample using samtools mpileup option and annotated and filtered with bcftools. INDELs were excluded. We set a minimum depth to 10x and a minimum map and base quality to 20. A total of 3,783 SNPs were detected in 59 candidate genes and 96 lncRNAs, and 64 of these SNPs were selected for being genotyped with the OpenArray Genotyping platform in 945 Iberian purebred pigs of the same Iberian line. Association analyses between these genotypes and IMF content were separately performed in 945 slaughtered pigs of the same Iberian line, implementing an animal model with the carcass weight, and the number of copies of the most frequent allele fitted as covariates, and the joint effect of Montanera x slaughter batch (33 levels) and the polygenic additive effect were included as random effects. As result of association analyses, a total of eight SNPs mapped on seven genes (ADIPOO, ANKRD1, ARID5B, EGR2, MLLT11, PVR and TRAFD1) and one lncRNA (ALDBSSCG000000055) have significant effects on IMF (nominal p-value < 0.05). The most relevant detected SNP was the rs709961585 located on ANKRD1 with an additive effect of -2.4 (0.7) on IMF % (mean =  $5.0 \pm$ 2.2), but just two different genotypes were observed. Although relevant SNP effects on IMF were detected in the current study, further analyses should be carried out before implementing MAS for IMF in this commercial line.

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Florence, Italy ABSTRACT

## Green Charcuterie project: effects of pig diets added with linseed and antioxidants on muscle gene expression and identification of differentially expressed genes

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One of the most relevant goals of pig industry is the improvement of the nutritional profile of meat by increasing the content of polyunsaturated fatty acids (PUFA) and, in particular, n-3 fatty acids. Nevertheless, PUFA level can worse the technological features of the meats due to an increase of oxidative events. In order to obtain meat products of high quality satisfying consumers and industry requests, 48 Italian Large White pigs (12 pigs/group) were fed with a control diet (D1, a standard finishing diet) and three experimental diets (D2 supplemented with linseed; D3 with linseed, selenium and vitamin E; D4 with linseed and polyphenol-rich plant extracts) plus. The animals were slaughtered at about 130 kg live weight and gene expression profile of muscle Longissimus thoracis (LT) was then analysed to study transcriptome changes related to diet integrations. The transcription profile of LT muscle was analysed using an Illumina RNA-seq approach. The differentially expressed genes (DEG) were considered significant with |Log<sub>2</sub>FC|.30 and FDR≤.05. Functional analyses of the DEG were carried out using Cytoscape and DAVID. The comparison of the muscle transcriptome between D2 and D4 showed that the genes up-regulated by D2 were involved in RNA processing, GPCR downstream signalling, RNA splicing via transesterification reactions. Some functions down-regulated by D2 vs. D4 were related to Muscle organ development, Response to elevated platelet cytosolic Ca<sup>2+</sup>, HIF-1 signalling pathway. Negative regulation of protein metabolic process. Some DEG detected in this research (PPARGC1A, PRKACA, PRKAR2A) are involved in glucose and energy metabolisms, both important for muscular and adipose tissue development and growth. The D4 showed to up-regulate a larger number of genes compared with D2, and most of them were involved in the regulation of transcriptional activity, the regulation of glucose metabolism, and the regulation of muscle tissue trophy and activity. The effect of polyphenols supplementation observed in D4 vs. D2 seems to be important for the improvement of muscle cells functionality and metabolism with increased glucose uptake by the muscle cells. These findings provide new knowledge on the effects of dietary plant extracts and n-3 PUFA on pig muscle transcriptome. Further investigation on the phenotypic effects in vivo and in derived meat products are needed also to address future studies to improve the pig diet formulation.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

Genomic inbreeding in European autochthonous pig breeds: analysis of

**ABSTRACT** 

## runs of homozygosity

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Conservation and management of autochthonous pig genetic resources are designed considering inbreeding. Inbreeding is traditionally calculated using pedigree information which is summarized in an inbreeding coefficient (FPED). However, FPED has several limits, some of them, are more important in local breeds in which many animals could have incomplete pedigree information, as mating events cannot be correctly registered in extensive production systems. The availability of commercial single nucleotide polymorphism (SNP) arrays in all main livestock species, including the pig, has opened new opportunities to estimate the inbreeding level directly using whole genome information. Runs of homozygosity (ROH) are chromosome regions that are homozygous at each polymorphic position. The ROH length and the genome proportion covered by ROH are good indicators of the age, origin and level of autozygosity and thus inbreeding. In this study we analysed ROH in a total of 1129 pigs from 20 European autochthonous pig breeds (Alentejana, Apulo-Calabrese, Basque, Bísara, Majorcan Black, Black Slavonian, Casertana, Cinta Senese, Gascon, Iberian, Krskopolje, Lithuanian indigenous wattle, Lithuanian White Old Type, Mora Romagnola, Moravka, Nero Siciliano, Sarda, Schwäbisch-Hällisches Schwein, Swallow-Bellied Mangalitsa and Turopolje; from nine different countries: Croatia, France, Germany, Italy, Lithuania, Portugal, Serbia, Slovenia, Spain) and three cosmopolitan breeds (Italian Large White, Italian Landrace and Italian Duroc). All animals were genotyped with the GGP Porcine HD Genomic Profiler v1 chip (68,528 SNPs). PLINK software was used to identify five size classes of ROH (1-2 Mbp, 2-4 Mbp, 4-8 Mbp, 8-16 Mbp and >16 Mbp). Then the genomic inbreeding coefficient, defined as the proportion of genome covered by all ROH and divided by the total length of autosomal genome (FROH), was calculated for each animal and averaged over all pigs analyzed for each breed (40-50 per breed). Alentejana pigs had the lowest averaged FROH  $(0.029 \pm 0.018)$  whereas Apulo-Calabrese had the highest averaged FROH (0.103  $\pm$  0.038). The highest FROH individual value was observed in a Black Slavonian pig (0.295). Apulo-Calabrese, Casertana and Sarda breeds had the highest number of ROH >16 Mbp, suggesting that their autozygosity was derived from recent ancestors whereas Italian Large White and Italian Landrace had a high frequency of short ROH, indicating a remote origin of common ancestors. This study contributed to understand the population genetic history of the investigated pig breeds and provided information that could be useful to manage these pig genetic resources.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

## Diet effect on adipose tissue transcriptome along growth in Iberian pigs

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Tissue composition largely determines the quality of meat and meat products and is influenced by factors as diet, genetic type, age or sex. Diet influences animal body and tissue composition by direct deposition or due to the bioactive effects of nutrients on gene expression. This influence is not well characterized and is known to be affected by factors including timing and physiological status. In this study, we evaluated the effects of diets supplemented with oleic acid or carbohydrates on tissue composition and gene expression along growth in Iberian pigs, with RNA-seq technology. Fifty-nine Iberian males started the dietary treatment at 19.9 kg of average live weight (LW) and were kept under identical management conditions and fed two different isocaloric and isoproteic diets provided ad libitum: HO diet enriched with 6% high-oleic sunflower oil and CH diet with carbohydrates as energy source. Twenty-nine animals were slaughtered after seven days of treatment, with 24.1 kg of average LW and thirty animals were slaughtered after forty-seven days of treatment, with 50.2 kg of average LW. In both slaughter groups, the diet induced changes in the fatty acid composition of ham subcutaneous adipose samples, which reflected the diet received, and indicated higher lipogenesis in CH diet, as expected. Twelve animals from each slaughter group were randomly selected for RNA-seq analysis (six corresponding to each diet). The diet effect on adipose tissue transcriptome was separately evaluated in the two slaughter groups. In the group of 7 days of treatment, we detected 171 differentially expressed genes (DEGs) (FC 1.5-33) conditional on diet while 207 DEGs (FC 1.5-21) were observed in the group of 47 days of treatment. Out of the DEGs observed at both stages only 39 were common and most of them showed an opposite regulation in the two slaughter groups. In the first stage, only 38 DEGs were up-regulated in HO diet while 133 were increased in CH diet. However, in the second stage 124 DEGs were upregulated in HO diet and 83 in CH diet. The results suggest that the diet effect differs between samplings depending on the length of the treatment, with potential interaction effects. We performed a functional analysis (GO and metabolic pathways enrichment) of the DEGs, which showed enrichment of biological functions related to response to stress, inflammatory and defense response, cellular lipid metabolic process and growth and tissue development in both groups. In addition, we detected enrichment of several metabolic pathways such as calcium signaling, actin cytoskeleton signaling, PI3K-Akt signaling and cytokine-mediated signaling pathway. The results indicate a relevant effect of the diet on transcriptome and suggest a slightly different response of adipose tissue gene expression along time, with DEGs affecting relevant functions and biological pathways.

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**SESSION 6** 

### NUTRITION AND REPRODUCTION

MAIN LECTURE

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ence, Italy

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### nce, Italy ABSTRACT

# Iberian pig as a model to study protein and lipid metabolism in fatty pigs

ABSTRACT

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The knowledge on nutritional requirements is crucial for a balanced nutrition of pigs. In this way, enhanced efficiency of nutrient utilisation, and economic, environmental and animal welfare benefits can be achieved. For an accurate assessment of nutrient requirements previous metabolic characterization is needed. This is the case of fatty pig breeds in which metabolic characteristics differ widely from those of conventional lean pigs. In the case of Iberian pig, which could be considered as a model for fatty pigs, applied nutritional and comparative physiology studies have been undertaken to elucidate their metabolic peculiarities. Some of these studies and their main results and implications will be described in this review. Protein deposition is lower and fat deposition much higher in Iberian pigs along their productive cycle compared with conventional pigs. Muscle protein synthesis and degradation rates are greater in Iberian pigs, leading to reduced protein accretion (g/d) and less body protein mass in comparison with conventional pigs. Therefore, energy cost of body protein accretion is higher in the native breed as both processes -protein synthesis and degradation-require considerable energy inputs (Nieto et al. 2012). Along with the higher protein turnover rates, higher relative weights of visceral tissues make the Iberian pig less efficient in the use of dietary protein and energy than leaner pig breeds. Therefore, a feeding system adapted to the particular metabolic needs of this native pig breed has been developed from weaning to slaughter (140-160 kg BW). Protein requirements, assessed by a direct method analysing animal growth and protein deposition responses to variable protein intakes have been defined using the maximum capacity to accrete protein (Pmax, g/d) and the marginal efficiency of protein deposition (response in protein deposition to changes in energy intake). In all these experiments the ideal protein concept (perfect balance of essential amino acids needed for maintenance and productive functions, NRC, 2012) was followed for formulation of dietary protein. The amino acid (AA) composition of body protein is considered constant, although some studies indicate that AA content of body proteins may be affected by factors like protein and energy intake, body weight, and essential AA supply. Pigs with marked differences in body composition and protein deposition rates -like fatty and lean genotypes- may differ in AA composition of body proteins. This hypothesis has been tested in Iberian and Landrace × Large-White pigs. The AA composition of carcass and muscles of both pig genotypes was assessed in young pigs of both types fed similar amounts of a lysine-adequate or a lysine-deficient diet with identical total crude protein and ME contents. Differences in carcass concentrations were found for some indispensable AA in which higher ratios to lysine have been observed for Iberian pigs. These findings could indicate relatively higher requirements of these AA for Iberian growing pigs In addition, fatty and lean pigs responded in general terms in a similar way to a deficient lysine supply, modifying the growth rate and modulating the protein composition of specific carcass components, a fact possibly related with their different body functions (Palma-Granados et al. unpublished).

The lipid content and fatty acid (FA) profile in pig tissues are influenced by genotype and nutrient supply with implications in meat quality. Selection for reduced back-fat thickness in pigs has resulted -at similar dietary FA supply- in lower total saturated (SFA) and monounsaturated (MUFA)

and higher polyunsaturated FA content (PUFA). A major factor affecting meat organoleptic characteristics is the amount and composition of intramuscular fat (IMF). Different nutritional strategies involving the manipulation of dietary protein have been proposed to increase IMF in pigs, although the effect seems to be linked to dietary lysine shortage. It has been reported that reduced lysine supply was effective in increasing IMF content in *longissimus* muscle of lean pigs but not in fatty pigs. However, we demonstrated that reduced lysine supply could be an effective strategy to increase IMF in muscles of both fatty and lean piglets maintained in similar nutritional and management conditions. Changes in biochemical and histological muscle parameters -composition of IMF and proportion of oxidative muscle fibers- when feeding a lysine deficient diet were also observed in agreement with others (Katsumata *et al* 2008). The effect of lysine deficiency on lipid metabolism was tissue-specific, with an activation of lipogenesis in muscles but no apparent stimulation in backfat adipose tissue (Palma-Granados *et al*. 2019).

There is an important issue related to animal welfare that concerns the surgical castration of male pigs to avoid boar taint in pork products. Immunocastration has been described as an effective strategy to prevent sexual development and odor of meat (Batorek *et al.*, 2012). This approach has great potential in pigs slaughtered at high weights, such as local pigs, although their long production cycles make necessary to develop specific vaccination protocols. Within the TREASURE project, adaptation of immunocastration protocols to Iberian pigs has been investigated, along with immunocastration effects on performance and on product quality. Our studies underline increased lean growth capacity of male pigs before effective immunocastration, although some detrimental effects on meat quality have been detected in *premontanera* male pigs (Seiquer *et al.* 2019). Further research in this area is needed.

The research program developed on Iberian pig nutrition fully support the concept of using feeding regimes adjusted to the particular needs of the growth period under consideration, as protein requirements suffer a dynamic change along the productive life of pigs. Regarding AA requirements, a refinement of diet formulation would be desirable in the future in light of recent findings on indispensable AA composition in lean tissues of Iberian pigs. On the other hand, suitable feeding protocols including lysine-reduced diets could be designed -tailored for each pig type in specific periods of the productive cycle- in order to increase intramuscular lipids without penalizing the growth of lean carcass components. Further research in this area is warranted to increase sustainability and profitability of Iberian pig production.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

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Seiquer, I., Palma-Granados, P., Haro, A., Lara, L., Lachica, M., Fernández-Fígares, I., Nieto, R.

SESSION 6

NUTRITION AND REPRODUCTION

ORAL COMMUNICATIONS

rence, Italy ABSTRACT

## Relationship between feed efficiency and meat quality in Iberian pigs

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The present study aimed to investigate the relationship between feed efficiency and meat quality on a commercial vs. a local acorn diet in Iberian pigs. A total of 30 Iberian pigs were provided consecutively, individually, with 4 kg/d of a regular concentrate diet for 30 days (Concentrate; 21.5% corn, 15% wheat, and 38.2% barley), and with 8 kg/d of acorns for 21 days (Acorn). After the Acorn period, all animals fed extensively on acorns, roots, and herbs for 19 days and were groupsupplemented with 4 kg/d/animal of concentrate diet (Montanera). Body weight was recorded at the beginning and end of each period. Body weight gain (BWG) during Concentrate and Acorn are direct measurements of feed efficiency. Animals were slaughtered two days after Montanera and meat quality was investigated in Longissimus dorsi: CIELAB lightness (L\*24), redness (a\*24), and yellowness (b\*24), the amount of myoglobin (Mb), centrifugal drip (Cdrip) and purge after thawing, cooking loss (CL), and shear force in raw (SFr) and cooked samples (SFc). Results show that only productive performance in Montanera was related to meat quality. Pigs with higher BWG in Montanera had a significantly lower CL (r = -0.50, P < 0.01), a\*24 (r = -0.57, P < 0.001) and Mb (r =-0.41, P < 0.05). The redness coordinate a\*24 was highly positively correlated with the amount of Mb (r = 0.68, P < 0.0001) and with CL (r = 0.60, P < 0.0001). Pigs raised outdoors have redder meat than those raised intensively resulting from exercise, and a change in muscle characteristics towards slow contraction, better vascularization, and more oxidative metabolism. It is thought that faster BWG in Montanera and more exercise is related to better taste of Iberian pork. Our results show that faster BWG in Montanera improved water holding capacity but may result in paler meat.

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Florence, Italy ABSTRACT

# Performance, carcass and ham traits of heavy pigs managed on different targets for age and weight at slaughter

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The Italian pig sector, mostly devoted to the production of Protected Denomination of Origin (PDO) dry-cured hams, must comply with technical specifications established by the PDO regulations. Weight and age at slaughter are key husbandry features in such production, with a minimum of 160 kg and 9 months, respectively, which have been established to attain an acceptable degree of maturity of meat. Changes in feeding strategies and in the genetic background of pigs may affect the appropriateness of the standard weight/age target in providing green hams with optimal dry-curing properties. This study investigated the effects of variation in age and/or weight at slaughter on live performance, carcass and green ham traits of finishing heavy pigs. A total of 112 C21 Goland pigs (58 gilts and 54 barrows) of 90 ± 10 kg initial body weight (BW) were allotted to the following groups (2 pens/group): i) pigs fed a ad libitum diet (10 MJ NE and 153 g CP per kg) and slaughtered at 170 kg, regardless of the age (AL170), or at 9 months of age, regardless of the weight (AL9M); ii) pigs fed a restricted diet (10 MJ NE and 122 g CP per kg) to reach 170 kg BW at around 9 month of age, which is the current target for PDO heavy pigs (RE9M); iii) pigs fed a restricted diet (10 MJ NE and 107 g CP per kg) to reach 170 kg around 9.5 months of age (RE9.5M). Feed intake, weight gain, carcass and cuts weights were recorded, and green thighs were measured for subcutaneous fat thickness and scored for ham characteristics. Data were analysed with a mixed model including pen as random effect and sex and group as fixed effects. Weight gain exceeded 0.9 kg/d in AL170 and AL9M pigs, whereas it was 0.74 and 0.64 kg/d in RE9M and RE9.5M pigs, respectively. As a consequence, the target weight of 170 kg was reached after 85, 113 and 127 days in feed for AL170, RE9M and RE9.5M, whereas AL9M reached a final BW of 194 kg. RE9M, the control group for the PDO condition, had feed efficiency comparable to ad libitum fed pigs, carcasses showing the lowest backfat thickness and the greatest incidence of loins and dressed hams, and green hams with the lowest marbling scores and dressing losses. Increasing the age at slaughter decreased feed efficiency and incidence of dressed hams on carcass, and increased marbling score, but not the subcutaneous fat thickness of hams compared to RE9M pigs. Ad libitum fed pigs showed the lowest uniformity in body, carcass and dressed ham weight, and the greatest subcutaneous fat thickness of hams. Beyond performance and carcass traits, manipulating weight and/or age of heavy pigs at slaughter can affect green hams suitability for drycuring.

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rence, Italy ABSTRACT

# Betaine increases portal appearance of branched chain amino acids in Iberian pigs

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Although several authors have suggested that oligopeptides (PAA) may be absorbed intact across the gastrointestinal tract and fulfil the protein requirement for growth and maintenance, their importance is often neglected. Furthermore, the idea that amino acids (AA) in pigs are exclusively absorbed from the small intestine and released in the bloodstream only in the form of free AA (FAA) has been questioned. The aim of this work was to determine portal concentration of branched chain AA (Isoleucine, Leucine and Valine) from PAA and FAA in Iberian pigs fed betaine supplemented diets. Eight 30 kg Iberian barrows were randomly assigned to dietary treatments and fitted with a catheter in the portal vein. The diets consisted of a control diet supplemented or not with 0.5% betaine. Barley and soybean meal-based control diet contained 101 g CP/kg DM, 4 g L-Lys/kg DM, and 14.7 MJ ME/kg DM. Blood samples were periodically taken from the portal vein at -30, 30, 60, 90, 120 and 180 min after feeding 1,200 g of diet and plasma was stored at -20°C until analysed for FAA and PAA. Plasma concentration of AA was analysed before and after acid hydrolysis (6 N HC1 at 110°C for 24 h) of samples first subjected to chemical deproteinization (adding 1:1 20% trichloroacetic acid) and physical ultrafiltration (3000 MW cutoff filter), and PAA were calculated as the difference between total and FAA.

Overall, while betaine supplementation increased portal concentration of branched chain AA as PAA (82, 58 and 94% for isoleucine, leucine and valine, respectively; P<0.001), portal concentrations of free isoleucine and valine decreased (23 and 12%, respectively; 0.01<P<0.001) and leucine was not altered (P>0.05). In conclusion, increased branched chain AA concentration from oligopeptides in the portal vein after betaine supplementation was consistent with a greater AA supply to liver and peripheral tissues.

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Florence, Italy ABSTRACT

# The Relative Roles of Birth-Weight and Postnatal Diet on Patterns of Growth and Fattening in Iberian Pigs

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In commercial lean swine breeds, the quality and economic profit of the final product is markedly determined by the birth-weight of the piglet. Piglets with low-birth-weight (LBW) have lower growth potential, lower feed efficiency and higher adiposity than normal-birth-weight piglets (NBW). The same has been described in Iberian pigs reared for fresh meat products (110 kg live-weight at 7 months-old). However, there is no information on traditional longer productive cycles for cured products (10-12 months old with 140-160 kg live-weight).

Therefore, the objective of the present study was to determine the relative roles of birth-weight (NBW vs LBW) and the postnatal diet (maintenance or MD vs fattening or FD) on the changes in body-weight (BW), average daily weight-gain (ADWG) and back-fat depth (BFD at the P2 point) in purebred Iberian pigs reared for around 13 months. Forty-eight Iberian piglets were chosen at birth; half of them were selected as NBW (n=24, 1.34±0.02kg of BW) while the remaining were selected as LBW (n=24; 0.84±0.02kg of BW; P<0.0001 with NBW). Half of the piglets in each group were females and half were males castrated at birth; males were heavier than females in both NBW (1.36±0.04 vs 1.32±0.03kg; P<0.0001) and LBW groups (0.85±0.04 vs 0.83±0.03kg; P<0.0001).

All the piglets were allocated together and fed with the same control diets from weaning to around 135 days-old. At that moment, NBW remained heavier than LBW (41.9±0.1 vs 34.0±0.1kg; P<0.0001), showing a significantly higher ADWG during this period (0.30±0.01 vs 0.24±0.01kg/day; P<0.0001). Males had a higher ADWG during this period and hence were heavier than females in both groups (P<0.0001).

Afterwards, the pigs were allocated to different diets from 135 to 385 days-old. Half of females and males from NBW and LBW had *ad libitum* access to a fat-enriched diet (6.3% of fat; group FD) whilst the remaining pigs were fed with a commercial diet fulfilling maintenance requirements (2.8% of fat; group MD). High fat intake causes significant differences in BW at 385 days-old between groups FD and MD (172.0±0.2 vs 119.3±0.2kg, respectively; P<0.0001), with a significantly higher ADWG from 135 to 385 days-old (0.54±0.02 vs 0.33±0.02 respectively; P<0.0001). The values for BFD were also higher in the group FD (7.2±0.04 vs 3.1±0.03cm in the group MD; P<0.0001). The birth-weight still affected BW since NBW were heavier than LBW both under the diet FD (179.3±0.3 vs 166.0±0.3kg, respectively; P=0.04) and MD (127.1±0.4 vs 111.3±0.4kg, respectively; P=0.03), but without sex-related differences. ADWG and BFD were not significantly affected by sex or birth-weight.

In summary, growth patterns of Iberian piglets were affected by sex, birth-weight and postnatal diet. However, the effects of sex and birth-weight were more evident during the first months of life. From 4 months of age, within either control or fattening diets, NBW and LBW piglets and female and male sexes developed similar ADWG resulting in similar final BW and BFD.

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ABSTRACT

# Effect of maternal antioxidant and omega-3 supplementation on the subcutaneous fatty acid profile of the Iberian offspring

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Western diet is deficient in omega-3 (ω-3) fatty acids (FA) while being abundant in omega-6 (ω-6) ones. Health-related concerns imply an increasing interest in improving the consumption of ω-3 FA and studying its metabolic implications. Pork is greatly consumed worldwide, and its FA composition can be easily changed. Also, maternal nutrition is essential in the development of the piglets, being possible to change the offspring fatty acid composition by using oils rich in ω-3. Since they are more susceptible to oxidation than ω-6 FA, the addition of antioxidants to the diet like hydroxytyrosol is necessary. Hydroxytyrosol has also shown to improve piglet growth and development, especially in big litters. The aim of this work was to determine the effect of the inclusion of linseed oil and hydroxytyrosol in the sow's diet during gestation in the fatty acid composition of the subcutaneous backfat of offspring. Twelve pure Iberian sows were inseminated with Iberian semen. In order to induce low-weight piglets, food was adjusted to fulfil the 50% of daily requirements on day 35 of pregnancy and linseed oil (4%) and hydroxytyrosol (0.1%) were added to the diet of the treatment group (7 sows) and non-supplemented animals (5 sows) served as controls. Thirty-six piglets (18 treated and 18 controls) were measured and weighted periodically until 2 months of age, when backfat samples were taken. The fatty acid composition of the inner (IL) and the outer layers (OL) of the subcutaneous fat were studied separately using gas chromography. Results showed an increase in ω -3 FA like C20:5n-3 (P<0.001) and C22:6n-3 (P<0.05) in both layers in treated animals, which could be explained by the higher activity of desaturases and elongases related to  $\omega$ -3 FA in the treated group (P<0.05). This can also explain the difference in the n6/n3 ratio (P<0.0001 in both layers, lower in treated animals). C20:4n-6 was increased in treated animals (P<0.001 in both layers), possibly because of the effect of hydroxytyrosol in ω-6 FA. Desaturation index (C18:1/C18) was also lower in the treated group in OL (P < 0.001), which could be related with differences in the activity of enzymes between animals and layers. MUFA/SFA ratios were also lower in the treated group in OL (P<0.05), which can be explained by the lower concentration of C16:1n9 and C16:1n7 in the treated group (P<0.05). In summary, the inclusion of  $\omega$ -3 with hydroxytyrosol in the sow's diet improves the litter fatty acid profile of the subcutaneous backfat, which has positive implications in human health.

#### Acknowledgement

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# Performance of lactating Iberian sows fed diets of different protein concentration

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X International Symposium of Mediterranean Pig

Lactating Iberian sows have lower body weight (BW) and smaller litter size than sows of conventional breeds; however, no specific systematic studies have been conducted to determine their nutritional needs. The aim of this work was to evaluate the effect of dietary crude protein (CP) concentration (of ideal amino acid composition) on in vivo performance of lactating Iberian sows Twenty-eight pure Iberian sows of Silvela strain from third to sixth parity were used. After delivery, they were fed one of 4 experimental diets (4 treatments, 7 sows / treatment with homogeneous distribution of parities). The experimental diets were: control (T1), low (T2), average (T3) and high (T4) CP concentration, providing 13% CP and 0.8% total lysine (Lys), 12.3% CP and 0.7% Lys, 13.9% CP and 0.9% Lys, and 14.8% PB and 1% Lys, respectively. Litter size was adjusted to 7 pigs by cross-fostering. Sows and litters were weighed after farrowing and at weaning. During lactation  $(28 \pm 2 \text{ days})$  piglets received only sow milk. Sow backfat thickness was measured after farrowing and at weaning by ultrasound equipment. Milk samples were taken from 6 sows per treatment in the middle of lactation. Blood samples were taken at farrowing and weaning to analyse blood metabolites and hormones. One piglet per litter was slaughtered at weaning for body composition analysis to calculate nutrient deposition during lactation. Data were analysed by ANOVA to identify the effects of sow dietary treatment on the parameters studied. There were no differences among treatments at farrowing for sow BW, backfat thickness, total piglets born and total piglets born alive (P>0.05). At weaning, there were no significant differences on sow BW loss and backfat thickness loss (P>0.05). No significant differences among treatments for plasma metabolites and hormones were detected, although plasma triglyceride concentration of sows at farrowing were higher than at weaning (P<0.05). Nutrient composition of sow milk was similar among treatments except for a trend towards higher fat content on milk of T1 sows (P=0.07). Litter growth during lactation and weight at weaning were higher in piglets from T1 and T4 sows (P<0.01). Carcass protein gain during lactation was higher in piglets from T3 and T4 sows (P<0.05) with no significant differences among treatments for fat, ash and water carcass retention (P>0.05). Although increasing dietary protein level in lactating Iberian sows did not show clear benefits on sow performance, there seems to be a positive effect on protein deposition in piglets. Further studies, increasing the number of animals, will help to clarify some of the findings of the present study. The provided data will contribute to increase the knowledge on nutrition of Iberian lactating sows from which there is little information.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

# POSTER SESSION

76

rence, Italy ABSTRACT

# Effects of a bioadditive based on *Lactobacillus* strain in weaning piglets

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This study was conducted to evaluate the effects of a biotechnological additive based on *Lactobacillus* spp. The status health animals and reduction of pollution from halls piglet's growth was followed. 60 piglets from Topigs hibrids, 40 days±3 days of age were randomly assigned to 2 homogeneous groups (control-C and E, 6 pens/10 piglets, 3 replicates/group) with a polyculture supplementation of 2%/kg feed. The feed was administered *ad libitum* in the flour form, 2 meals/day. The bio productive performance, body weight (BW), average daily gain (ADG) shows significant differences between the groups: final BW was 18.268 kg in C vs. 19.775 kg in E, with an ADG of 0.355 kg/day in C vs. 0.533 kg/day in E. The addition of additive involves a reduction of *Enterobacteriaceae* from faces piglets with 25% in E. *Lactobacillus* spp. additive was able to reduce the number of *E. coli* from E group, when in C was recorded an increase of almost 40%. The lactic acid bacteria additive can be used in weaning piglets, represents a natural alternative for maintaining the normal functionality of intestinal tract, the zootechnical performances was performed too.

X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

# The cost of Iberian Pig in extensive conditions: A case study of different marketed products according to Spanish regulations

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The sector of Iberian Pigs in Spain has experimented several changes in the last years due to the Quality Standard regulation approved in 2014. According to this regulation, all pig breeders and fatteners must adapt their productive system depending on the product they market (black, red, green or white label). In this sense, calculating and knowing the unit cost of the different type of pig products makes decision-making and management for producers much easier, improving both, their productive activity and the efficiency. However, most producers of Iberian pigs do not have information about costs.

In this sense, the aim of this paper is to calculate and to analyze the cost of the different products that the quality regulation allows under two types of labeling: red (acorn feed fattening) and green label (fodder fattening) both in extensive conditions; identifying which are the costs in which the farmer has greater possibilities of management. For that purpose, we apply two different cost models: the *Full Cost* and the *Direct Costing*, that allows the classification of costs in direct and indirect costs and variables and fixed costs. We have defined two costs of center: "*Recria*" and "*Montanera*", to impute the indirect cost in the model.

The main results show that the cost is higher with higher level of quality, in both models Full and Direct Costing. The cost of the red label pigs is higher than the green label pigs. The red label profitability ratio is near to 50% comparing to the green label which is around 37%. However, the fixed cost of the red label is higher than the green label, which is explained because the red label pigs feed natural resource comparing to the green label which feed cost is considered a variable cost. By type of cost, feed is the higher component in both models, but the cost of the piglet represents a 25% of total cost of each type of animal. In this sense, the main cost that the breeder could be manage is the feed and the purchase of the piglet for fattening, because these two costs represent more than 50% of the total and variable cost per unit.

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# Autochthonous Portuguese Alentejo Local Pig Breed (Sus ibericus, SANSON, 1901): Preliminary Scientific Notula II

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Within the Portuguese autochthonous pig breeds, the Alentejo local pig breed (Sus ibericus, SANSON, 1901), according to our objectives outlined, defense, protection and promotion of the local pig breed and its emerging row, the R&D project of Alentejo local pig breed, in this second scientific notula, we only indicate the standard of the nipple variety of the breed, stressing the sustainability of the Mediterranean ecosystem, not neglecting the sui generis quality of the final products, meat of Alenteio local pig breed and certified hams (PDO and PGI). The methodology used to carry out the work of the aforementioned research project, we collect data on farms of Alentejo local pig breed producers (currently 180 producers), registered with the Alentejo Pig Producers' Association (ACPA), for analysis and interpretation of results, as well as information on annual exhibitions in morphological competitions of breeders in shows. By analyzing the data and results obtained, in the genotype of the nipple variety, in the decade (2009-2019), we verified that the official entities only limited themselves to approving and transcribing the standard, for the Genealogical Book of the Alentejo Swine Breed, recording the effect of the "reproductive goodness "of the nipple boar we have already published. Also, we find that there are no explicit references, neither to the nipple variety nor to the genotype blonde, as recommended by the EEC / European Union, since 1984, taking into account the common ancestral trunk of the Iberian pig. Data and results collected during the international competition, 35th. Ovibeja2018, in Portugal, through the morphological competition of the breed under study, there is evidence of crosses between the lines (black or *caldeira* and blonde or auburn) already observed by us in 2015. Thus, we concluded by recommending the elaboration of more systematized (R & D) projects and works, taking into account the different areas of the knowledge economy, within the various sectors of the above mentioned emerging row, particularly in the scientific area of bioinformatics in genotyping. But in this case, only if there are methodologies with expeditious techniques, that is, capable of combating possible fraud in the various sectors of the emerging row, protecting the said genotypes from the danger and imminent threat of extinction of the existing lines and varieties, particularly those mentioned above, and as their respective high end products for export.

#### Acknowledgement

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## **Environmental impacts of contrasted Iberian pig production systems**

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X International Symposium of Mediterranean Pig

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The Iberian pig production in Spain is characterized by three types of livestock management systems: montanera (outdoor system and fattening with only natural resources on large surfaces), cebo campo (outdoor system and fattening with compound feed and natural resources in a small surface) and cebo (indoor conventional system). The aim of the present study was to perform an environmental evaluation by Life Cycle Assessment of the three Iberian pig production systems in Spain. Data were collected from 3 farms (one of each type) through surveys about farm characteristics and management. The environmental impacts considered were Climate Change (CC), Acidification (AC), Eutrophication (EU), Cumulative energy demand (CED) and Land Occupation (LO). These impacts were calculated at the farm gate and expressed per kilogram of live pig. The analysis was performed with Simapro software (version 8.5.2.0). CC impact showed lower value in montanera (3.43 kg CO<sub>2</sub> eq), intermediate value in cebo campo (5.52 kg CO<sub>2</sub> eq) and higher value in cebo (7.53 kg CO<sub>2</sub> eq), mainly because of lower impacts associated to feed production. AC impact was equal between cebo campo and cebo (0.13 molc H+ eq) while montanera (0.09 mole H+ eq) was lower due to a different manure management. EU impact was similar between montanera and cebo (0.046 and 0.044 kg PO<sub>4</sub><sup>3</sup> eq, respectively) while cebo campo (0.07 kg PO<sub>4</sub><sup>3</sup> eq) was higher CED impact was higher in cebo campo and cebo systems (38.67 and 32.69 MJ, respectively) due to the greater amount of compound feed needed to fatten pigs while in montanera (20.88 MJ) was lower. LO impact in montanera (38.31 m<sup>2</sup> year) was higher than in cebo campo and cebo systems (13.83 and 11.18 m<sup>2</sup>.year, respectively) because of natural resource dependence. Among the production stages which contribute the most to environmental impact, the farms with animals fattened with compound feed (cebo campo and cebo) show a similar contribution of their production stages to environmental impacts. The stage with greater contribution was growers owing to a long productive period and dependence on compound feed. Followed by fatteners due to dependence on compound feed but on a shorter period. The lowest contribution is the production of piglets. In montanera, the growing period was the major contributor in CC, AC, EU and CED. In addition, production of piglets was the second contributor and fatteners show the lowest contribution in this system. Fatteners montanera contribution was higher in LO impact because of high land use due to dependence on natural resources (acorns and grass) in the finishing period. In conclusion, montanera is the Iberian pig system with the lowest environmental impacts on account of independence of compound feed in the fattening period due to the use of natural resources in the dehesa.

#### Acknowledgement

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X International Symposium of Mediterranean Pig

Florence. Italy ABSTRACT

# Optimization of Iberian pig extensive systems during the growing period: use of alternative protein sources through grazing

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The growing period in traditional Iberian pig production is characterized by outdoor systems where the animals are fed concentrate and natural pasture. This extensive production system should be improved through new feeding and management strategies to optimize the economic and environmental sustainability, as well as the animal welfare. This study aimed to evaluate grazing of nascent triticale (Tn; with high protein content at this stage) as a sustainable alternative protein source during the growing period in Iberian pig outdoor systems. Triticale has a high re-growth rate and therefore can be grazed and later harvested for grain. Pure Iberian immunocastrated male pigs were distributed in 3 groups during 48 days as follows. Control pigs (Ctrl; n=16) were fed standard concentrate (c) in a natural pasture plot, whereas Tn+c pigs (n=16) were fed the same concentrate in a nascent triticale plot. To test a more sustainable approach, which might also be used to prolong the growing period if needed, Tn+g pigs (n=14) were fed unground triticale grain in another nascent triticale plot. Plot surface areas were approximately 5 hectares/group. Concentrate and triticale grain daily rations were 3.125 kg/animal. Grain ration was equated to concentrate rations to partially compensate for undigested grain losses, whereas most of the protein intake in the Tn+g group was expected to come from triticale pasture. Body composition echography and blood sampling were performed to assess body condition and metabolic changes. Pasture was collected for nutrient content analyses. At the beginning of the study  $(9.3 \pm 0.03 \text{ months of age})$ , live weight (LW) of the 3 groups was the same (96.1  $\pm$  1.3 kg; p = 0.905). After 48 days, Tn+g had a lower (p=0.027) LW than Ctrl and Tn+c (108.32, 125.10 and 125.41 kg, respectively). This was likely due to the lower digestibility of the unground triticale grain, as whole grains were easily observed in Tn+g faeces. There were no differences in growth among the 2 concentrate fed groups. Triticale pasture had higher protein and lower fibre and lignin contents than natural pasture. If economically feasible for a given farm, triticale grain should be crushed to minimize faecal losses. In conclusion, preliminary results suggest that triticale grazing during the growing period may be an interesting sustainable feeding strategy, firstly because triticale grain could generate an economic benefit (grain and straw sale) or could reduce costs by using triticale grain on animal feed in the same farm, and secondly because it implies a reduction of economic and environmental impacts by decreasing the dependence on concentrate and on imported soy as a protein source.

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# Growth description of Iberian x Duroc crossbred pig through the evaluation of nonlinear mathematical models

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Growth modelling in pig production has been widely used and can provide us with information on management issues such as appropriate slaughter age, health conditions, age of sexual maturity and genetic potential. The objectives of this study were to adjust a non-lineal model (NLM) to the growth of Iberian x Duroc crossbred pig. The NLM evaluated were Logistic, Bertalanffy, Gompertz and Polynomial 3rd order. The growth data were obtained from Iberian x Duroc crossbred pig with ad libitum feed in two separate experiments. Experiment 1 (n=15); the pigs from 38 days of life (9.43 kg live weight (LW)) to 227 days (151 kg LW) and in experiment 2 (n=17); the pigs start with 47 days of life (11.58 kg LW) to 273 days (157.8 kg LW). Weight control was performed 7 to 10 times during this growth period. The growth parameters estimated were: gm: maximum animal net growth (kg), ti: time at inflection point (days), wi: weight at inflection point (kg), vm: maximum net growth rate (kg days), t100; time at 100 kg (d), t160; time at 160 kg (d) and w300; weight at 300 d (kg). The analyses were carried out with the NLIN procedure of SPSS. The coefficient of determination (R2), root mean square error (RMSE), Akaike's information criteria (AIC) and Bayesian information criterion (BIC) were used to determine the best growth model. The Polynomial equations showed a better adjustment with estimations, in exp 1 and exp 2, of vm: 0.924 kg·d and 0.861 kg·d, ti: 163 d and 178 d, wi: 96.5 kg and 90.1 kg, t100: 167d and 190 d, t160: 240 d and 278 d and w300: 184 kg and 166 kg, respectively. However, Polynomial equations have no biologically interpretable parameter and therefore may not be the most suitable choice for describing growth patterns. Towards the end of life, the polynomial models do not well represent the asymptotic shape of the curve so they are bad predictors for the future and cannot give us for example adult weight values. The Gompertz model is very close to the polynomial model, but with the advantage of providing parameters with biological interpretation. In this model, gm was 253.1 kg and 240.4 kg in exp 1 and exp 2, respectively.

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Relationship between several live animal measurements and carcass quality in Iberian pigs

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The high added value in the breeding of Iberian pigs is directly related to the performance of their noble pieces, especially hams, shoulders and loins. Thus, in farm it is interesting to know the weight of these in the live animal, although it is difficult to do it manually with morphometric measurements, since it increases the handling and animal stress. Alternatively, fast and non-contact techniques are increasingly used, such as image analysis or ultrasound. So, the aim of this study was to develop a predictive model of the weight of the ham in the live animal using image analysis to extract the morphometric measurements and ultrasounds to know the thickness of the backfat. A total of 49 male and female IberianxDuroc crossbred castrated pigs were used in this study. Pigs were reared under free-range system montanera in a farm in Extremadura, Spain. Measurements of ultrasonic backfat depth in the last rib and images were taken the day before slaughter at 150.05  $\pm$ 11.11 kg of live weight. Two cameras were used to take images of each pig -top position and lateral position, which were used to measure length and width of pigs respectively. These were taken while the pigs were eating in a handling cage, since pigs remained in a relatively stable position. On the other hand, manual measurements of length, perimeter, backfat thickness and weight of the ham were carried out in the slaughterhouse. Correlations between these measurements and ham weight were studied. Length, perimeter and fat thickness showed a significant correlation with ham weight (p<0.001). The final model showed  $R^2 = 0.7582$  and standard error of estimate (SEE)= 0.4912. Subsequently, the relationship between manual measurements (cm) and these taken in vivo through image analysis (cm) was studied: pig height (82.59  $\pm$  3.22) with ham length (69.38  $\pm$  2.15), width pig  $(29.84 \pm 1.19)$  with ham perimeter  $(72.98 \pm 2.54)$  and fat thickness -through ultrasound-  $(4.98 \pm$ 0.48) with ham fat thickness (3.57 ± 0.53), which showed a  $R^2 = 0.711$ , 0.410 and 0.321 and SEE= 1.749, 0.925 and 0.4, respectively (p<0,05). So, in order to predict the weight of the ham from measurements taken in vivo, a multiple regression was carried out between in vivo measurements and ham weight. Fat thickness measured by ultrasound was removed because it was no significant. and therefore the variables pig height and width pig (p<0.001) were used. The final model showed  $R^2 = 0.6026$  and SEE= 0.6228. It can be observed that the use of non-contact techniques in vivo decreases the reliability of the predictive model with respect to post-slaughter measures but would be an alternative to predict the weight of the ham in vivo.

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X International Symposium of Mediterranean Pig 16-18 October 2019 Florence, Italy

ABSTRACT

# Group housing of sows: Effects of season and day of grouping on sow welfare and reproductive performance

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A decrease in reproductive performance was observed by some farmers during summer, following the entry into force of Directive 2008/120/EC requiring sows and gilts to be group housed during pregnancy. Mixing sows at 28 days of pregnancy might increase both embryo resorption and abortions due to the adverse effects of cortisol on corpus luteum, therefore some farmers asked the Public Veterinary Services the authorization to group sows after 35 days from service. Our aim was to assess the effects of mixing at different gestational ages on sows' hormonal profiles and reproductive performances. Two batches of sows (one during winter and one during summer) were followed during a reproductive cycle (PIC Camborough-22, N=30 sows per batch, average parity: 4.4±2.8). Each batch was split into two groups: one was kept in single gestation stalls until day 28 of pregnancy and then moved to group housing (MIX28), the other was kept in single stalls until day 42 of gestation and then moved to group housing (MIX42). Group housing was on partiallyslatted floors with ad libitum fibrous feed. Bristles were sampled from each sow at the end of pregnancy (when they were moved to farrowing rooms) and bristle cortisol was assayed as an indicator of cortisol levels during the entire pregnancy. Blood samples were drawn from 12 sows per group, at 32 (B1) and 46 (B2) days after service to assess progesterone plasmatic concentration. Productive and reproductive parameters of all sows were recorded. Plasmatic progesterone and hair cortisol values were submitted to two-way ANOVA, using season and day of mixing as the main effects.

Hair cortisol at the end of pregnancy did not differ between MIX28 and MIX42 sows (5.53 vs 5.98pg/mg; P<0.60). Progesterone level as well did not show any significant difference between MIX28 and MIX42 sows at 46 days after service (B2) (23.18 and 21.76ng/ml respectively; P<0.41). However, at 32 days after service MIX28 sows had lower progesterone levels if compared to MIX42 (25.15 vs 30.14ng/ml; P<0.01), probably due to a transitory effect of mixing (cortisol) on corpus luteum. A significant (P<0.001) effect of season on plasmatic progesterone was observed, with lower concentration during summer than during winter at both blood samplings, regardless of the day of mixing (26.54 vs. 29.50ng/ml at B1; 18.71 vs. 25.70ng/ml at B2). All progesterone concentrations fell within the physiologic range for pregnant sows.

No differences were observed between seasons and between groups in the main productive and reproductive parameters (gestation and lactation duration, newborns, stillborns, mummified and weaned piglets, abortions, etc).

Our results show that a prolonged stay (42 days) in single stalls does not increase stress levels of sows (no differences in cortisol from bristles). On the other hand, such a prolongation does not improve productive or reproductive performances. Our findings therefore do not confirm the necessity (as expressed by farmers) to keep sows in single stalls until at least 35 days after service.

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## Woodland and livestock systems in inland areas of Sardinia: the case of the native pig breed

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The forest area covers more than half of the Sardinia surface (24,089 km<sup>2</sup>) and about 5,000 km<sup>2</sup> are covered by oak-groves. About 2,224 km<sup>2</sup> of the woodland areas of Sardinia is managed by the regional forestry Agency named FoReSTAS. About 40% of these is public concession of common lands where FoReSTAS ensures to the local populations the traditional uses like the practice of grazing and the harvest of wood and acorns. Swine is raised in Sardinia since the ancient Neolithic and persists in the reason of the pasture availability in the large woodlands areas. However, many of those rural areas are facing depopulation due to outmigration and negative natural population growth, so the population ageing is one of the most significant demographic and social trends that negatively affect also the agricultural activities. Since 2006, the autochthonous Sarda swine breed has been officially recognized and the relevance of this autochthonous pigs is rather limited both in terms of total pigs registered and farms. The persistence of the African Swine Fever (ASF) virus in Sardinia represents one of the most relevant serious constraint for the development of swine industry based on autochthonous pigs reared in the woodlands. For this reason, the promising achievement of the ASF eradication plan is identified as the element of trust in the future of the swine industry of Sardinia. The FoReSTAS Agency have planned a program aiming to encourage the development of farms rearing autochthonous pigs in woodland in conformity with the biosecurity procedures appropriate to protect farm animals from ASF. This project is going on according to the measures made by the "Project Unit for the eradication of African swine fever in Sardinia". This pilot-project is resulting from the collaboration between the FoReSTAS Agency, some local administrations and the swine farmers concerned. At now, the project involved four relevant municipalities (Alà dei Sardi, Urzulei, Talana and Seui) of the mountain areas of Sardinia. With the project some woodland areas were assigned, to local farmers, by calls for tender following publication of a contract notice. In some cases the entire acreage of a pasture-lot is reserved for a single farmer, while in other ones a large pasture-lot has been assigned to joined farmers. The mean acreage of the pasture-lot is about 7-10 hectares for each farmer. The main commitments for the

- to pay the annual rent for the pasture-lot:
- to make the border enclosure of the pasture-lot with a double wire mesh fence at least 150 cm high and at least 80 cm away from each other:
- to not exceed the regional livestock stocking density which is limit fixed at 0.15 tons of live weigh per hectare.

In conclusion, the participatory approach used by this pilot-project seems effective to ensure that community members and farmers were positively involved in a program aiming to improve the sustainability of swine production in woodlands of Sardinia.

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**ABSTRACT** 

### Production traits of Banija spotted pig

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The aim of the work is to present Banija spotted pig regarding breeding activities, rearing system, and most important production traits. Banija spotted (BS) pig is a local Croatian breed recognized as the third and the youngest Croatian autochthonous pig breed at the end of 2018. The breed was developed at the end of the 19<sup>th</sup> century by crossing between local Turopolje pigs, Berkshire, and later by improved pigs in Landrace type. The breed was very popular in Banija (or Banovina) region in Central Croatia, especially for the processing of meat in dry-cured products, and in the past meat from BS pigs was the main raw material for the famous local meat industry. The breed was almost disappeared at the end of the 20<sup>th</sup> century due to a pressure of highly productive imported breeds and as a consequence of war activities in the 90s in this region. A few years ago. professionals from the Faculty of Agriculture, Faculty of Veterinary Medicine, and Ministry of Agriculture, supported by local authorities, started to work on revitalization and protection of the population of BS pig. They visited farms with pigs of BS type and started to register animals in the herdbook. During this period, the main goal of breeders was to increase the number of purebred animals, and also to determine the most important production traits of the breed. Nowadays, the breed is still rare and under risk with the population around 80 sows and 20 boars. The traditional production system of BS pigs is outdoor or semi-outdoor with farrowing and piglets rearing stage mainly indoor, and using outdoor area for sows and fattening pigs, usually from spring to late autumn. Production traits of the breed are in general moderate. Litter size is approximately 7 to 8 piglets born alive. Heavy fatteners have average daily gains between 300 and 500 g, depending on the production system and level of feeding. Usually fattening pigs reach final weights of 150 kg or more in the age of 16 months. Keeping fattening pigs outdoor with appropriate feeding regime ensures slower growth, higher meat percentage, desired carcass composition, and good meat quality. Slaughter traits of BS fattening pigs showed high processing value of the breed. Regarding meat quality traits, preliminary analysis showed favourable values of pH, meat colour, and water holding capacity, with no prominent defects. Additional research is needed on other meat quality traits, as well as on branding of specific local products from the meat of BS pigs.

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Effect of feed restriction and sex on growth performances and carcass composition in "Porc Negre Mallorquí" pig breed

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The "Porc Negre Mallorqui" (Majorcan Black Pig-MBP) is a native breed from Mallorca island, characterized by its high rusticity and fat depot, and devoted to the production of traditional fat-rich products such as "sobrassada". The objective of this work is to analyze the effect of sex and feed restriction on growth, feed efficiency, fat depot and carcass traits in order to evaluate the putative impact on "sobrassada" production. A total of 68 MBP animals were divided in two groups (17 castrated males and 17 females per group) and fed either *ad libitum* or under 24.67% restriction a similar diet with a barley and peas mixture (14% protein and 1,7% fat). Weight at the beginning of control period was 99.5 kg on average (SD=1.8) in both groups; no weight differences were observed between sexes. Animals fed *ad libitum* showed higher growth rate than animals under restriction. Conversely, animals under feed restriction reported a better feed conversion ratio (FCR.kg/kg 5.55 vs 6.84). In general, castrated males showed significantly higher growth rate (475 gr/day vs. 415) and lower FCR than females. Regarding carcass characteristics, females showed higher loin depth (36.0 mm vs. 32.6) and lower back fat thickness (34.158±1.6 mm vs 39.5±1.6) than males. The outer fat layer increased by 2%, the middle layer decreased by 0.8% and the inner layer decreased by 0.5% in animals fed with restriction compared to pigs fed ad libitum.

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prence, Italy ABSTRACT

### Forage chain management in rearing system of Cinta Senese

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The aim of this study was to evaluate the effect of two different feed managements for Cinta Senese pig breed in order to limit the use of commercial mixture. A total of 34 castrated male pigs, 18 Cinta Senese and 16 Large White X Cinta Senese, were used. Animals were equally allotted within each genotype in two feeding systems; pasture or pen. Animals reared on pasture were fed following the forage chain with a supplement of commercial mixture whereas animals reared in pen were fed only commercial mixture. The amount of mixture for pastured animals was calculated based on the presence of the different grazing sources in each trial period. The animals were weighed once a month. Data were analysed by ANOVA using breed and rearing system as fixed effect and slaughter weight as continuous effect. Also, interaction breed\*rearing system was tested but it never attained significance. At the end of the trial (278 d) pen groups ate a total of 627 kg/head of commercial mixture against 490 kg/head of pastured animals. At slaughtering no differences were found between groups for average daily gain; as regard genetic type, Cinta Senese pigs showed higher percentage of fat cuts, and lower of lean cuts than crossbreed (total fat cuts, 25.50 vs 23.30% and total lean cuts, 69.91 vs 71.61% respectively). The greater adiposity of pure breed was also confirmed in the sample join (portion of loin including subcutaneous fat) (P<0.05). Physical and chemical analyses were not different between genetic types. As regard the rearing system effect, the use of forage chain would seem to satisfy the nutritional requirements of the animals which present similar performance to animals in pens.

In conclusion, the farm forage chain could be an optimal strategy for Cinta Senese pig rearing as it could lead to a reduction of the use of commercial mixture without affecting both animal performances and carcass quality, allowing to extend the grazing period and, consequently, reducing feed costs.

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ABSTRACT

# Identification of differentially expressed key genes of *Longissimus* lumborum samples from Portuguese Alentejano and Bísaro local pig breeds

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Most of the swine industry nowadays is about productive and profitable fast-growing lean breeds raised under intensive conditions leading to meat and fat rich in saturated fatty acids. The Portuguese local Alentejano (AL) and Bísaro (BI) pig breeds present high intramuscular fat (IMF) content which contributes to highly appreciated pork products. These breeds have different ancestors; AL belongs to the Iberian group, presenting lower growth rates and higher lipid accretion and unsaturated fatty acids level when compared to BI, which belongs to the Celtic group, sharing ancestors with leaner breeds such as Large White and Landrace. The goal of this work was to compare the muscle gene expression profiles of AL and BI pig breeds to better understand the influence of the genetic background in the main metabolic processes occurring in the Longissimus lumborum (LL) muscle, namely in terms of lipid synthesis, muscle tissue formation, protein synthesis and cell proliferation. LL samples were obtained at slaughter, from adult AL and BI pigs with ~150kg body weight. Total RNA was extracted and sequenced for a transcriptome comparison analysis. A total of 250 genes were found to be differentially expressed (DE) in LL samples (q<0.05) conditional on breed, with 174 DE genes up-regulated in AL  $(log2(fold\ change) = 0.65$  to 7.03) and 76 in BI (log2(fold change) = 0.63 to 4.53). Genes related to skeletal muscle development and function, such as MYH3, MYH13 or ACTN4, were significantly up-regulated in BI when compared to AL, which is in agreement with the higher muscle mass of the former breed. Genes involved in lipid metabolism were up-regulated in AL, including SCD (q=0.05), responsible for catalysing the reaction that introduces the first double bond into saturated fatty acyl-CoA substrates, which agrees with the higher unsaturation of fat tissues generally associated with the former breed. A functional enrichment analysis (metabolic pathways and GO enrichment) was performed for the DE genes and the identified functions included tissue development, cellular growth and proliferation, quantity of connective tissue and lipid metabolism. Potential regulators found that explain the observed gene expression changes in the dataset included molecules such as: ADORA2A, CEBPA, SMAD3 and PPARG (predicted to be activated in AL); HDAC and ASXL1 (predicted to be inhibited in AL).

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# SNP association analyses for myoglobin content and water holding capacity in a purebred Iberian pig population

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The traditional Iberian pig production system Montanera linked to the genetic characteristics of the breed, have greatly influence in meat quality. In a previous study, a panel with SNPs mapped on candidate genes for meat quality traits was designed and relevant effects of SNPs located on CAPNI, CASP9, PRKAG3 and CTSL genes on meat tenderness, instrumental color, thawing and cooking losses measured on Iberian purebred pigs were observed. The objective of the current study was to analyze the effects of the SNP panel on myoglobin content (MB) and centrifuge force water losses (CFL). A total of 32 SNPs were genotyped on 565 Iberian purebred individuals using TaqMan® OpenArray® Genotyping Plates and additive effects were estimated through univariate animal models with QXPAK software. Four out of the 32 SNPs were fixed in the typed individuals and one showed genotyping problems. The other 27 had a MAF>0.05, and nine of them showed intermediate frequencies. Nineteen SNPs were used for association analyses since the remaining eight co-segregate. The association analyses revealed significant effects of SNPs mapped on CASP9 and PRKAG3 on the myoglobin content and mapped on CAPN1, CTSL and PRKAG3 on CFL. The most relevant effects were observed for CASP9 rs32430761 on MB (a= 0.05±0.02) and for CAPN1 rs81358667 on CFL (a= -0.68±0.20). In the previous study, a significant effect of CASP9 rs34618816 were observed on (a\*) instrumental color parameter (redness), which is related with myoglobin content and CAPN1 rs81358667 SNP significantly affected tenderness and water losses traits, which are related with centrifuge force water losses. Although further analysis should be performed, these SNPs could be used for selecting sires in a selection scheme for meat quality traits.

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ABSTRACT

# Carcass and meat quality of purebred Black Slavonian pigs and crossbreds with Duroc

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The aim of the study was to investigate the influence of breed and sex on carcass and meat quality traits of purebred Black Slavonian pigs and their crossbreds with Duroc. The study included 30 purebred Black Slavonian pigs and 30 crossbred pigs with Duroc (both experimental groups with equal number of gilts and barrows) reared under extensive keeping conditions. Feeding was based on cereal mixture (50% corn, 30% barley, 10% oats, 10% soybean) supplemented with alfalfa. During the winter period, pigs were fed alfalfa hay. When pigs reached predetermined slaughter weight of approximately 130 kg, they were transported to the commercial abattoir and slaughtered after stunning with CO<sub>2</sub> (90%). At the slaughter line following carcass traits were measured: carcass weight, carcass lengths, muscle and backfat thickness, surfaces of LD (longissimus dorsi) muscle and belonging backfat, as well as ham length and circumference. Meat quality traits measured included pH values and electric conductivity (at m. semimembranosus and LD muscle), meat colour (L\*a\*b\*), cooking loss and instrumental tenderness. Crna slavonska purebreds had significantly higher backfat surface, as well as ham length and circumference. Breed did not influence other carcass traits. Purebred Black Slavonian pigs had higher pH<sub>45</sub> values in LD muscle and pH<sub>24</sub> values in SM muscle than crossbred pigs. Also, they exhibited lower L\* values and higher a\* values in SM than the crossbreds. However, the experimental groups did not differ in L\* values measured in LD muscle, while crossbreds had higher a\* values in the same muscle compared to purebred animals. Significant effect of sex was found for carcass length (both a and b), and ham length and circumference, where barrows exhibited higher values in all traits.

The results of the study indicate that crossing the Black Slavonian pig with Duroc does not compromise meat quality of the crossbred animals, however it does not improve its carcass traits when reared in extensive system.

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# Changes in the genetic status of Crna slavonska pig breed over 15 years

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The purpose of the present work was to establish the genetic status of Crna slavonska pig breed over last 15 years based on analysis of genetic parameters from microsatellite (MS) data.

20 Crna slavonska pig samples were collected in the year 2004 in the eastern part of Croatia (Slavonia county). In that year, the number of registered pigs of Crna slavonska breed was 562 sows and 57 boars. For genetic analysis 18 MS markers were used: SW24, SW857, SO225, SO227, SW240, SO215, SO218, SO005, SW122, SO155, SO226, SO090, SO178, SW911, SO002, SW951, SW632 and IGF1. Number of alleles per locus, observed and expected heterozygosity and inbreeding coefficient ( $F_{is}$ ) were calculated using Genetix 4.02 version.

Other 50 samples of Crna slavonska pigs were collected in the year 2013, when the number of registered sows was 839, and boars 120. The interest in breeding of this autochthonous pig breed is still growing, so the number of registered pigs in the year 2017, when the last 131 samples were collected, was 1930 sows and 242 boars. The samples from years 2013 and 2017 were analysed with 25 MS markers: S0026, S0155, S0005, Sw2410, Sw830, S0355, Sw24, Sw632 Swr1941, Sw936, S0218, S0228, Sw240, Sw2406, Sw122, Sw857 S0097, Sw72, S0226, S0090, Sw911, S0002, Sw2008, Sw1067 and S0101. Cervus 3.0.7. software was used to calculate observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity at each locus and number of alleles per locus ( $N_A$ ). Inbreeding coefficient ( $F_{IS}$ ) was calculated using Genetix 4.02 version.

Results for the year 2004 show, that  $N_A$  was 5.6,  $H_E$  was 0.649,  $H_O$  was 0,592 and  $F_{is}$  0.116. The MS data analysis for the year 2013 revealed following results:  $N_A$  7.08,  $H_E$  0.665,  $H_O$  0.632 and  $F_{is}$  0.062. For the year 2017, the results were as follows:  $N_A$  7.24,  $H_E$  0.67,  $H_O$  0.642 and  $F_{is}$  0.053, respectively.

Analysis of the MS data revealed that despite of the quick expansion of the Crna slavonska breed in the last 15 years, heterozygosity levels and mean number of alleles per locus is higher in the years 2013 and 2017 in comparison with the year 2004. Also, in quickly growing populations it would be expected, that the inbreeding coefficient will grow with time, but our results show opposite - the  $F_{is}$  values dropped from 0.116 in the year 2004 to 0.053 in the year 2017. A part of this discrepancy could be explained by the fact, that in the year 2004, 18 MS markers were used for the genetic analysis, while later analyses were performed with 25 MS markers. Growing heterozygosity levels and dropping  $F_{is}$  values are also in line with our previous findings on persistence of genetically "non-black" (melanocortin receptor 1 heterozygous) animals in Crna slavonska pig breed, which both suggest uncontrolled introduction of commercial pigs into this autochthonous pig breed. Further admixture studies, including commercial pig breeds, are needed to confirm this hypothesis.

ABSTRACT

### Muscle transcriptome in Mangalitsa and Moravka pigs

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Mangalitsa (Swallow-Bellied strain) and Moravka are two Serbian local pig breeds with some relevant phenotypic differences. Mangalitsa are fatty pigs with high meat quality, while Moravka has combined productive abilities, both for lean and fat, and has a higher share of meat compared with Mangalitsa breed. The aim of this study was to compare the L. dorsi muscle transcriptome between both breeds in order to achieve a better understanding of their metabolic differences. Pigs used for the present study were slaughtered at 12 months of age, at an average live weight of 116 kg for Mangalitsa (MA), and 131 kg for Moravka (MO). Muscle samples of 10 surgically castrated male pigs were obtained. 5 corresponding to MA and 5 to MO. RNAseq analysis was carried out and standard Tuxedo Protocol was employed for the data analysis. Transcriptome data analysis showed 195 differentially expressed genes (DEGs) between two breeds (99 overexpressed in MA and 96 overexpressed in MO). Fold changes (FC) ranged from 1.73 to 11.42 in MA and from 1.69 to 42.77 in MO. The functional analysis of DEGs carried out with Ingenuity Pathways Analysis software showed the activation of functions related to lipid metabolism (concentration of lipid and triacylglycerol), inflammation and immune cell trafficking in MA; and enhanced organism and tissue development-associated functions in MO. Some key genes involved in lipid synthesis were upregulated in MA, such as PDK4 (FC=9.67) or FABP4 (FC=4.33), in agreement with the higher fat content observed in L. dorsi muscle in this breed. Several relevant canonical pathways were activated in MO, including Interleukin signaling, Growth Hormone signaling or Integrin signaling, which could be related to higher growth rate and muscle tissue development observed in MO pigs. Potential regulators and causal networks were predicted to underlie the differential gene expression data. Among them, CEBPB and IFNG were predicted as main regulators affecting lipid metabolism differences between the two breeds. The analysis done provides a relevant information regarding the molecular basis for differences in phenotype observed in the analyzed breeds and highlights specific candidate genes and regulators potentially involved in some of the main pork production and quality traits.

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# Optimal contribution selection: a tool for sustainable management of Banija spotted pig

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Breeding programmes for conserved breeds of domestic animals are often oriented to conservation role, although selection for economically important traits in such breeds can be beneficial for the sustainable breeding. Best linear unbiased prediction (BLUP) is the appropriate method for selection in local and autochthonous breeds. However, conservation and selection seem to be opposite breeding goals and optimisation between maintaining genetic diversity and genetic gain is required. For that purpose, tools within optimal contribution frame (OCS) are available. The litter size was the focal trait for the application of the OCS on the Banija Spotted (BS) pig population. Genetic diversity parameters were estimated based on pedigree data including 751 available records and single nucleotide polymorphism (SNP) genotypes for 24 animals. Genotyping was carried out using GP Porcine HD 70 K array. The genomic inbreeding coefficients (F<sub>ROH</sub>) were calculated as the proportion of the autosomal genome covered by runs of homozygosity. Trends of effective population size were estimated from linkage disequilibrium as implemented in SNeP software. Data set contained 224 farrowing records for BS sows. Genetic parameters and breeding values for litter size were estimated using a repeatability model for litter size with herd, mating season, and boar as fixed effects, and permanent environmental and additive genetic effects as random effects in the model. Evolutionary Algorithm (EVA) software was used to estimate pedigree-based genetic diversity parameters of the population and to apply optimal contribution selection in the population of Banija spotted pig. Average pedigree-based inbreeding coefficient in the population was 3.68%. Inbreeding rate per generation ( $\Delta F$ ) in the population based on genealogical analysis was 2.62% and effective population size was 19.06. Genomic inbreeding was 18%, while historical effective population size obtained from molecular information was 54. According to the obtained genetic parameters, Banija spotted pig belongs to the breeds with low genetic diversity. Estimated heritability for the litter size was 0.05. The predefined rate of inbreeding was restricted to 1% in order to optimise the increase of inbreeding and genetic gain. The application of OCS showed that genetic gain of the similar level can be achieved while restricting the increase of  $\Delta F$  to 1% in the population. As the restriction on the inbreeding rate is relaxed, the number of selected pigs decreases and the maximum contribution for a single selected male and female increases, resulting in the higher genetic gain. The Banija spotted pig population is suitable for a management strategy which jointly optimizes the response to selection and the levels of variability and inbreeding. Further steps include full integration of the molecular information from the microsatellite and SNP markers in the optimum contribution selection procedure.

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Genetic analysis on Vietnamese native pigs: variations of coat colour and porcine endogenous retrovirus genes

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Pig domestication is considered to have occurred in several various regions in the Eurasian continent. Vietnam has been expected to be one of them and reported to own 26 local breeds with distinctive appearances. In this study, we aimed to (1) analyze genetic relationships among Vietnamese native pigs (VNP) using genome-wide DNA markers (i.e. single nucleotide polymorphisms: SNP) and sequence variants in a coat color affecting gene and to (2) characterize VNPs by analyzing porcine endogenous retrovirus (PERV) gene. For these genetic analyses, we collected 90 samples from 15 breeds of VNPs from 15 distantly separated areas of the country and 18 samples from western commercial pig breeds. Genomic DNA was extracted and genotyped using Porcine SNP60 v2 Genotyping BeadChip and used for Sanger sequencing analysis of melanocortin 1 receptor (MC1R) gene. In addition, the copy number of PERV in the genome was measured by a real-time PCR-based method and the differences in copy number of PERV genes between pig breeds were conducted by Tukey's multiple comparison tests. ADMIXTURE analysis based on the SNP genotyping data revealed that VNPs were divided in three major clusters according to their geographic origin (i.e. northern, central and southern regions). In particular, the result of principal component analysis using the SNP genotyping data showed that VNPs from northern mountain area would have own genetic structure in each village compared with those from the other regions. In relation to that, the VNPs in northern region were classified into various coat colors such as 1) Black, 2) White, 3) Black/White, 4) Brown, and 5) the other. We identified three novel and non-synonymous SNPs in MC1R gene. Of those SNPs, two were specifically found in Brown coat color VNPs. Finally, the copy number of PERV genes in the genome estimated that most of the VNP breeds showed less than half PERV copies of those in western commercial pig breeds. Especially one breed of VNP in the northern area showed very low and stable mean copy number of PERV genes.

In conclusion, the results of this study firstly shed light on the distinct genetic features in terms of the genomic architecture characterized by the genome-wide DNA markers. Secondary, the novel SNPs identified in the *MC1R* gene may be available for animal forensic use to distinguish particular breed type. Moreover, VNPs are promising biomedical models for xenotransplantation studies considering the low level of PERV genes in some populations.

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ABSTRACT

# Technical validation of longissimus dorsi transcriptomic expression on purebred Iberian pigs divergent for meat tenderness from a RNAseq experiment

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Tenderness is a trait with a high relevance in meat quality. More tender meats are more desired by the consumer than tougher ones. Several authors have detected SNP effects on tenderness mapped in candidate genes as CALPASTATINE or CALPAINE1. Therefore, tenderness is a trait susceptible of being included in breeding programs. One approach for finding candidate genes consists in the assessment of differential expressed genes on individuals divergent for a particular trait. In a previous study, we analyzed the transcriptome of longissimus dorsi from individuals divergent for tenderness and detected 72 genes and new isoforms differentially expressed using RNA-seq technique. Although this approach has a high technical reproducibility, validation of results using RT-qPCR has been advisable. In the current study, we quantify the expression of seven differentially expressed genes through RT-qPCR. A total of 13 RNA samples from seven animals with softer meat (S group) and six with tougher meat (T) were used to carry out the technical validation of seven genes (MSTN, ANKRD1, FOS, COL1A1, MX1, ELOVL6 and NOS2) both differentially and not differentially expressed. Quantification was done using SYBR Green Mix in a LightCycler480, following standard procedures and data were analyzed with LightCycler480 SW1.5 software. Genorm software was used to calculate the stability of the endogenous genes and data normalization was performed using the normalization factors of B2M and ACTB genes. Pearson correlations between RNA-seq and RT-qPCR expression values and concordance correlation coefficient (CCC) were calculated to perform technical validation. A total of four genes (ANKRD1, FOS, COL1A1 and MXI) showed Pearson correlation values higher than 0.7 and CCC value was equal to 0.454. Pearson correlations corresponding to MSTN, ELOVL6 and NOS2 were lower than 0.7 and these results could indicate that we did not measure the expression of the same isoform of those genes in RNA-seq and RT-qPCR approaches. Therefore, further technical validation of more genes will be carried out.

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Lactic acid and glycogen contents, pH and meat quality from Iberian pigs raised on free-range and fed on acorn and grass: early, middle and late "montanera"

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Traditionally Iberian pigs have been free-range reared fed on acorn and grass in a system called "montanera". It is of great importance to know the effect of productive characteristics on the meat quality of Iberian pigs. *Post-mortem* glycolysis phenomena induce the accumulation of lactate in muscle tissue causing a rapid decline in muscle pH and affect ultimate pork quality. The aim of this work was to evaluate the effect of temporality (early, middle, late) of montanera, a traditional production system based on acorn and grass and free-range rearing of Iberian pigs, on pH, glycogen (GLY) and lactic acid (LA) at 45 min and 24 h *post-mortem* of m. *L. dorsi* and their effects on meat quality.

Pure Iberian pigs (n: 42) were randomly allotted in three batches corresponding to three "montanera": 1. Early "montanera" (November 1<sup>st</sup>- December 31<sup>st</sup>) -**EarMont**- (n: 16), 2. Middle "montanera" (December 1<sup>st</sup>-January 31<sup>st</sup>) -**MidMont**- (n: 12) and 3. Late "montanera" (January 1<sup>st</sup>-February 28<sup>th</sup>) -**LatMont**- (n. 14). Pigs were slaughtered at ~160 kg live weight. Pigs from different "montanera" were slaughtered in the same slaughterhouse following identical conditions of sacrifice. At 45 minutes and 24 h *post-mortem* m. *L. dorsi* were sampled and the pH, [LA] and [GLY] contents were assayed. At 24 h *post-mortem*, water holding capacity (WHC) and instrumental colour were determined. ANOVA was used to evaluate the differences between temporality of "montanera".

"Montanera" temporality did not affect pH values neither at 45 min and nor 24 h post-mortem in m. L. dorsi. pH values showed a slightly or inexistent decline at 24 h post-mortem. At 45 min post-mortem, [GLY] (mmol/kg muscle) was significantly higher in EarMont and MidMont than in LatMont; however, no differences in [GLY] were found at 24 h post-mortem. At 45 min and 24h post-mortem, [LA] (mmol/kg muscle) were significantly lower in EarMont and LatMont than in MidMont, increasing from 45 min to 24 h post-mortem. [GLY] decreased while [LA] increased from 45 min to 24 h post-mortem. Nonetheless, this increase did not reflect a drop in pH values. Meat quality traits were significantly affected by the type of "montanera". Therefore, WHC were significantly higher in EarMont and MidMont than in LatMont. Instrumental colour coordinates were significantly affected by temporality of "montanera", Cie L\*-value was significantly higher in EarMont and LatMont than in MidMont, meanwhile CIE a\*-value was not affected.

In conclusion, months of "montanera" in which Iberian pigs are fattened had a determinant effect on *post-mortem* changes in muscle and meat quality in terms of metabolism of glycogen and pH and meat quality.

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ABSTRACT

# Modelling preference heterogeneity and willingness to pay for Iberian dry-cured ham

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The aim of this paper is modelling preference heterogeneity and willingness to pay for Iberian drycured ham. For this aim, a choice based conjoint experiment was applied to analyse consumers' preference, being the attributes evaluated the following: Percentage of Iberian Breed, Type of Feed, Origin, Type of packaging and Price. Data were obtained through tasting sessions in Extremadura (SW of Spain). Participants were first required to evaluate the attributes of Iberian dry-cured ham and after the sensory test, they were given detailed information regarding the different categories of Iberian dry-cured ham and were asked to complete a choice experiment task.

The empirical analysis uses a mixed logit model, also called a random-parameters logit. This approach allows that the parameter associated with each observed variable to vary randomly among customers, without showing the restrictive "independence for irrelevant alternatives" property, which are limitations of the standard logit model. In this sense, we can obtain a range of values for the coefficients to measure the heterogeneity of the consumers' preferences and their willingness to pay for each attribute of the Iberian dry-cured ham. Another advantage of this model is that it is not restricted that the different levels of the attributes have a normal distribution. This is especially important when price is considered as an attribute in the model and allows to consider another type of distributions like the lognormal. The main results show that the standard deviations of the coefficients are significant, thus indicating that the parameters vary within the consumers and that, therefore, preferences for Iberian dry-cured ham are heterogeneous. On the one hand, the levels of attribute relative to the type of feeding present higher variety within the consumers. On the other hand, the type of package and the origin present low standard deviations, hence, the values are close to the estimated means. Finally, the price allows estimating the willingness to pay and its variations. The consumers present a high willingness to pay for acorn-fed Iberian dry-cured ham, as compared to the mixed pasture-fodder fed and fodder-fed Iberian dry-cured hams and have the highest standard deviations. The rest of the levels present low variations of willingness to pay, especially the type of package and the origin.

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ABSTRACT

# Alentejano and Bísaro pigs and their crosses: genotype effect on loin traits

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Alentejano (AL) and Bísaro (BI), the two main Portuguese local pig breeds, cohabited in Ribatejo region for some time, but their crosses disappeared in the 1950's. Although appreciated, no scientific data is available regarding the Ribatejano (RI) pig (ALxBI or BIxAL) animals or their meat. In order to assess the meat quality of RI pigs, castrated male pigs from AL, BI, ALxBI and BIXAL genotypes were studied within the framework of the TREASURE project. Raised in a traditional free-range system and fed commercial diets ad libitum, ten pigs from each genotype were slaughtered at ~65 (Trial 1) and nine at ~150 kg live weight (LW) (Trial 2). Longissimus lumborum (LL) physical-chemical traits (pH<sub>II</sub> at 24 h, intramuscular fat (IMF), myoglobin and total collagen) were measured and data statistical analyses were performed by one-way ANOVA. In both trials, BI, ALxBI and BIxAL attained slaughter weight faster, presenting higher ADG than AL pigs, but this difference was only significant (P<0.001) in trial 1 (65 kg). At 65 and 150 kg, % commercial yield was higher (P<0.01 and P<0.05, respectively) in BI than AL pigs, mainly due to heavier loin (P<0.05) and ham (P<0.001) weights in BI pigs. RI crosses presented intermediate values of those presented by the pure genotypes. Conversely, fat cuts (P<0.01) and backfat thickness (P<0.001) were higher in AL than in BI pigs in both slaughter weights, with RI crosses presenting again intermediate values. Physico-chemical data showed that AL pigs presented LL muscle with higher pH<sub> $\mu$ </sub> (P<0.01) (trial 1 - 5.57 vs 5.44; trial 2 - 5.72 vs 5.49) and IMF (P<0.05) (trial 1 - 6.7 vs 5.5; trial 2 - 6.9 vs 6.0), higher myoglobin (P<0.05) (trial 1 - 0.76 vs 0.54; trial 2 -0.86 vs 0.45) and lower total collagen (P<0.05) (trial 1 - 13.9 vs 17.1; trial 2 - 13.1 vs 16.7) content than BI. This last parameter influenced meat tenderness, with lower Warner-Bratzler shear force values in AL than BI pigs (data not shown). As to RI pigs, overall they presented LL with intermediate pHu, slightly lower IMF (more pronounced in trial 1), and identical pigment content and tenderness than the ones of AL pigs. In conclusion, RI pigs presented intermediate traits between the fatter (AL) and the leaner (BI) genotypes. These first results on RI pig's LL traits, suggest their potential for the production of heavier loins than AL pigs, with identical rich colour and tenderness, and high quality dry-cured products. RI pigs could therefore be an alternative to the use of other breeds on commercial crosses, helping to increase the income of local pig producers. and also maintain or increase the pure breed populations, contributing to animal biodiversity.

#### Acknowledgements

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### **Effects of High Pressure on Properties of Meat Products**

X International Symposium of Mediterranean Pig

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Cured pork loin and "Chourico de carne", both traditional ready-to-eat (RTE) meat products, from Iberian and Alentejano pigs, were processed by a cured drying stage followed or not by smoked stage, are characterize by the use of red pepper and garlic. This study aim was to evaluate the effect of high-hydrostatic pressure (HHP) process (600 MPa/8 min) and posteriours storage at 4<sup>0</sup> and 20 °C for 6 months in the meat products quality. The analyzed parameters along the storage period were: microbiological analysis – Mesophile aerobics, Lactic bacteria, S. aureus, Salmonella and L. monocytogenes, E. coli, C. perfrigens, coliforms and molds and yeasts; physical-chemical analysis - Instrumental color (L\*a\*b\*), lipid oxidation and protein oxidation; sensory descriptive analysis meat color, smell intensity, undesirable smell, hardness, juiciness, salty taste, sour taste, sweet taste, spicy taste, taste intensity, cured aroma and rancid aroma. In the microbiological analysis of "chourico de carne" after HHP, it had been shown the lowest mesophils count at 20 °C. The lactic bacteria had significant differences (p<0,01) along all storage in the cured loin, however the yeasts and molds had only differences at 90 and 180 days. E. coli, C. perfrigens, Salmonella and Listeria had presented results within the recommendable limits (<1 log UFC/g) as well as the S. aureus (<2 log UFC/g) in both RTE meat products. In the color parameters, temperature had shown influence in cured loin at 180 days in a\* and b\*, however HHP had influenced L\* at both temperature. In "chourico de carne", a\* had shown differences at day 0. With reference to lipid oxidation, the cured loin had presented significant different values with HHP at 180 days. In HHP "chouriço de carne" protein oxidation had shown differences at 20°C. However, neither the temperature nor the treatment had influenced lipid oxidation. In terms of cured loin sensory analysis, neither by temperature, nor by the treatment nor even by the storage time had affected the color, nevertheless smell intensity had been affected by all those factors. The hardness had been affected just by storage period, which was also the main factor to influence the decrease in sweet and spicy taste. Cured aroma had significantly higher scores at 90 days and rancid had shown differences at 20 °C without HHP. In relation to "chourico de carne", color had not been affected along the study, however the smell intensity had been by all factors (temperature, HHP and storage time), hardness was only affected by storage period and sour, spicy and sweet taste had not been affected by HHP but by storage presenting a decrease in scores along time. Also, cured aroma had significant differences along time, but rancid only had with storage time. Overall, this study suggested in some quality parameters the suitability of this novel approach for commercial adaptation, namely in microbiologically safety since it had presented for a long period of storage, levels of pathogenic below the expected ones.

Can raw thighs classified as E be suitable for the production of PDO hams?

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The aim of the present work was to collect information at the slaughterhouse on the relationship between carcass composition (lean meat content assessed according to the EUROP classification grid) and the quality of the raw thighs. Quality was assessed based on indicators included in the product specifications for Parma ham: trimmed weight, thickness of the fat layer (measured vertically at the head of the femur -best end-), presence of fat at the "coronet" (visually assessed), iodine number, linoleic acid content (C18:2).

The research included 11 slaughter plants. In each plant, 10 slaughtering batches and about 20 raw thighs per batch were selected. All pigs assessed had a carcass weighing more than 110 kg, in agreement with the definition of heavy pig. Overall, 2126 raw thighs were evaluated. At least 25% of the selected raw thighs in each batch derived from a carcass classified as E in the EUROP grid (F-o-M classification). Out of them 32.6% were classified as E, 35.5% as U, 24.7% as R and 7.1% as O. In 6 slaughtering plants, from each slaughtering batch we collected 10 samples of subcutaneous fat (including both inner and outer fat layer), of which 5 from raw thighs belonging to the E class and 5 from raw thighs belonging to the other classes (U, R, O). In order to assess if the fat from the raw thighs classified as E is more frequently non-compliant (iodine number > 70; C18:2 > 15%) with Parma ham production rules than U-R-O thighs, iodine number analysis was carried out on the subcutaneous fat samples, while the gas chromatography analysis of fatty acid composition is presently in progress. Trimmed weight, fat thickness and iodine value were analyzed with one-way ANOVA using the EUROP grid (E vs URO) as the main effect

As expected, despite the similar (P>0.48) weight of the trimmed thighs (14.525 kg for E vs. 14,486 kg for URO thighs) the thickness of the fat layer was lower (P<0.001) in thighs from E class than in thighs from URO classes (24.29 vs. 32.67 mm). Iodine value was significantly lower in thighs from URO than in thighs form E classes (65.62 vs 67.48; P<0.001).

Our preliminary results also show that the majority of the raw hams classified as E are compliant with Parma ham production rules for fat thickness (83.1%), fat at the "coronet" (97.6%) and iodine value (for this parameter only 73.9%).

If the compliance observed will be confirmed also by the gas chromatographic analysis (C18:2 content), it would be interesting to follow class E thighs also during the dry-curing process, to assess the final quality of the hams. It is reasonable to expect that, if the animals' diet is formulated to keep the iodine number and the linoleic acid content within the range prescribed by the production rules, the majority of thighs classified as E could be suitable for the long curing process.

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ABSTRACT

# Effect of immunocastration on chemical content and fatty acid composition of fat tissue of Mangalitsa pigs

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The objective of this research was to study the effect of immunocastration on the chemical content and fatty acid composition of fat tissue of Swallow-Bellied Mangalitsa pigs. Pigs were reared in facilities with the open-air section and fed a complete feed mixture adapted to their growth stage (from 25-60 kg of live weight (lw), mixture 1: 14.7% of crude protein and 13.6 MJ metabolisable energy ME/kg; from 60-120 kg of lw, mixture 2: 13.0% of crude protein and 13.5 MJ ME/kg). The study included 23 male pigs (11 immunocastrated and 12 surgical castrated pigs). Within each litter, one male pig was assigned to immunocastrated group and one to the surgical castrated group, at about 26 kg of live weight and 171 days of age. Immunocastration was performed using the Improvac® vaccine (first vaccination was conducted 85 days, and the second 40 days prior to slaughter). Pigs were slaughtered at 362±21 and 363±20 days of age and 121±24 kg and 116±8 kg of live weight for immunocastrated and surgical castrated group, respectively. Samples of subcutaneous fat tissue were taken in the area of the last rib. Effect of the treatment was evaluated by applying the GLM procedure and statistical significance of differences between treatment groups was determined by t-test. Surgically castrated pigs had greater average back fat thickness than immunocastrated pigs (65.1 vs. 53.3 mm, average of measurements at three different levels at carcass midline, P<0.05). Back fat tissue of immunocastrated pigs had higher water (5.85 vs. 4.36%, P<0.01) and protein (1.79 vs. 1.30%, P<0.001) contents than back fat tissue of surgical castrates, whereas fat content was higher in surgical than immunocastrated pigs (90.07 vs. 84.66%, P<0.05). Back fat tissue of Mangalitsa immunocastrated and surgically castrated pigs had the following fatty acid composition: 43.9 and 43.8% saturated fatty acids (ΣSFA), 43.5 and 44.2% monounsaturated fatty acids (ΣMUFA) and 12.6 and 11.9% polyunsaturated fatty acids (ΣPUFA), but differences were not significant (P>0.05). Ratio n-6/n-3 was 1.36 and 1.23 for immunocastrated and surgically castrated group, respectively (P>0.05). In conclusion, the castration method had an effect on the thickness and chemical content, but not on the fatty acid composition of the back fat tissue of Mangalitsa male pigs.

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V ABSTRACT

# Potential use of near infrared reflectance spectroscopy (NIRS) to predict the commercial categories of pre-sliced and packaged Iberian dry-cured ham

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Dry cured products from Iberian pigs are widely accepted by consumers due to their sensorial and nutritive attributes. They are regulated by National Quality Standard where several commercial categories are defined. In recent years, the use of new sales formats has increased, such as slicing and packaging. The traceability of these products is necessary, as well as the labelling according to the existing commercial categories (RD 4/2014). Thus, a thorough control by conventional methods online could increase the economic cost.

The use of non-destructive technologies has been widely described in previous studies for classification in different commercial categories. Therefore, the main aim of this study was to evaluate the effectiveness of the near infrared reflectance spectroscopy (NIRS) technology for the classification of in the different commercial categories established.

Ninety pre-sliced and packaged Iberian dry-cure ham samples of different commercial categories according to Quality Standard (RD 4/2014, Spain) (White, Red and Black labels) were selected (n=30 per group) and modified atmosphere packaging (MAP) (70% N<sub>2</sub>: 30% CO<sub>2</sub>) in commercial size of 100gr. These samples were measured with LabSpec 2500 (ASD inc. ®) coupled to Hi-Brite Contact Probe® to collect the NIR spectra (1000-2500nm) with IndicoPro® (Spectral Adquisition Software). Partial least square discriminate analyses (PLS-DA) algorithm was used to create a reliable predictive model for each category, where X variables were spectral data and Y variables were the commercial categories (White, Red and Black labels). The software used was The Unscrambler®10.2 of CAMO Software AS (Norway). Cross-Validation was used to validate the predictive model in each case. Determination coefficient (R<sup>2</sup>) and standard error in cross-validation (SECV) were selected to evaluate the reliability of prediction equations. The results showed an excellent behaviour in prediction model for White label samples ( $R^2$ =0.92 and SECV= 0.12), which was correctly classified with respect to Red and Black samples. However, there was greater similarity between Red and Black samples associated their productions conditions, and thus predictive models were less reliable ( $R^2 = 0.45$ ; SECV=0.37 and  $R^2 = 0.56$ ; SECV=0.36, respectively) than White labels samples equation.

In conclusion, NIRS technology could be used to classify the *White* label samples of the others. However, reliable NIRS equations were not found to classify *Red* and *Black* samples.

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ABSTRACT

# Changes in tissue fatty acid composition during postnatal development in Iberian pigs

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Tissue fatty acid (FA) composition has important implications in meat quality and metabolism in fatty pigs. Regarding metabolism, changes in cell membrane lipids (Polar lipids, PL) among others have consequences in cell functionality and molecular processes that ultimately can affect development and meat quality. In relation to meat quality, intramuscular fat (IMF) determines organoleptic characteristics and features with implications on human health, particularly in storage lipids (Neutral lipids, NL), which represents  $\approx 70\%$  of IMF. However, there is scarce data on changes during postnatal development, especially in non-adipose tissues. Hence, the goal of this study was to assess the effects of age on the FA composition of *Longissimus dorsi* IMF, and liver and brain fat in pigs at 25 and 180 days-old. Seventeen piglets (6 females, 11 males) and 20 pigs (9 females, 11 males) from the same litters were slaughtered at 25 (weaning) and 180 days-old, respectively. After slaughter, the tissues described above were sampled. The profile of FA was analyzed and identified by gas chromatography after fractionating FA into the main lipid fractions (NL and PL).

Our results showed a different FA profile associated with age in both lipid fractions of the three tissues with some coincidences. Only liver PL and NL fractions had the same changes in FA induced by age with higher saturated FA (SFA; P<0.01) and monounsaturated FA (MUFA; P<0.01), and lower polyunsaturated FA (PUFA; P<0.01) in older pigs than in piglets. In both brain lipid fractions, age decreased SFA (P<0.01) and increased  $\Sigma$ n-3 FA (P<0.01). On the other hand, both IMF lipid fractions showed lower desaturation index (C18:1/C18:0; P<0.01) and higher  $\Sigma$ n-6/ $\Sigma$ n-3 FA ratio (P<0.01) in older pigs than in piglets. In NL fractions across tissues, older pigs had higher MUFA (P<0.01) than piglets, probably due to feed FA content. However, older pigs showed a greater desaturation index (MUFA/SFA; P<0.01) than piglets only in both viscera NL fractions. Regarding sex, few interactions with age were found that could be due to the prepuberal stage of females. Both older males and females showed greater SFA in liver NL (P<0.01), desaturation index in brain NL (MUFA/SFA; P<0.01) and  $\Sigma$ n-6/ $\Sigma$ n-3 FA ratio in IMF PL (P<0.01) than younger males and females, respectively. In conclusion, tissue FA composition changes during postnatal life both in storage and cell membrane lipids with a weak sex effect before ending the prepuberal phase.

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ABSTRACT

# Tracing autochthonous pig breeds with meat near-infrared spectra data pig

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Near Infrared spectroscopy (NIRS) offers an easy to use and cost-effective tool for quantitative and qualitative application in animal science. Of special interest could be considered the use of NIRS for tracing meat origin on the basis of breed specification. The aim of this study was to investigate the potential use of meat NIRS as a "fingerprint" of autochthonous pig breeds. The research considered intact and grounded sample of Longissimus Dorsi (n=371) collected from 11 European local pig breeds from the TREASURE project, namely: Alentejana (ALE), Bisara (BIS), Crna Slawonska (CRN), Gascon (GAS), Iberian (IBE), Krskopolje (KRS), Lithuanian Wattle (LIA), Lithuanian White (LIH), Negre Mallorqui (NEG), Schwabish Hallisches (SCH) and Turopolje (TUR). For each muscle sample, two aliquots were scanned using FT-NIRS Antaris II model (Thermo Fisher Scientific) in absorbance mode considering the infrared region (3999 to 9999 cm<sup>-1</sup>) and averaged.

Discriminant analysis of principal components (DAPC) on meat NIRS was used to assess: i) breed traceability and ii) similarity among breeds. DAPC was applied on standardised (centred and scaled) spectra using the R package. For breed traceability, cross-validation was applied: five samples per breed were sampled at random, without replacement, and used in validation. The procedure was repeated ten times and each breed was analysed separately. In this case, all breeds were present in the training set. Similarity among breeds was assessed by excluding each breed from the training set and assigning the samples in the validation set to the breeds in the training one. Overall correct classification was 68.0 and 77.6% for intact and grounded meat, respectively. Alentejana had 100% correct classification for both intact and grounded meat. For CRN, KRS, LIA, LIH and NEG use of grounded meat spectra resulted in higher classification rates from 44 to 64% for intact and from 66 to 90% for grounded meat), while for GAS the opposite was found (80% for intact vs. 70% for grounded). For the rest of the breeds slight or no differences were observed between intact and grounded samples and classification rates ranged between 66 (CRN and SCH) to 72% (BIS). The lowest classification rates were observed in both cases for CRN. Similarity among breeds was greatly varied upon dataset used (e.g. ALE samples were classified as IBE (40%), TUR (40%) and SCH (10%) using intact meat while 100% were classified as CRN using grounded meat). Our results mark NIRS as a promising tool for traceability of pig breed meat origin and support the use of grounded over intact samples.

### Acknowledgements

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# Evolution of the volatile compounds along curing in Semimembranosus muscle of the Toscano ham

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The volatile profile of Toscano hams from 0 to 18 months of ripening was studied. Ten green hams of 15.60 were seasoned according to the 'Toscano' PDO Consortium ripening protocol. Semimembranosus muscle was sampled after trimming (0), 1, 3, 6, 12, 14, 16 and 18 months of ripening by means of 5-mm punch corer. The volatile compound profile was obtained by SPME-GC-MS using an Agilent 7890 Chromatograph equipped with a 5975A MSD with EI ionisation and a three-phase DVB/Carboxen/PDMS 75-um SPME fibre, exposed in the head space of the vials at 60 °C for 30 min. The identification of volatile compounds (VOCs) was done matching the peak spectra with library database and matching Kovat index (KIS) with KIS from literature. VOCs evolution was studied using a linear model and a multivariate approach, which was performed to test the feasibility of using the VOCs profile to predict the seasoning stage of Toscano ham. Ninetyseven VOCs, belonging to 7 chemical families (29 aldehydes, 16 esters, 14 alcohols, 13 hydrocarbons, 12 ketones, 10 acids, 2 furans and 1 nitrogenous compound), were identified. Firstly, the stepwise discriminant analysis (SDA) was applied to selected 26 compounds able to discriminate low maturing class (LMC) from high maturing class (HMC). Then, canonical discriminant analysis (CDA) was applied using the 26 selected compounds. The two maturing classes resulted clear separated (P < 0.0001). Among the 26 compounds the most influencing were: the 2,3-dimethyl pentane, acetophenone and 9-decenoic acid for LMC and dodecanoic, benzeneacetaldehyde, 3-octen-2one and pentanoic acid ethylester for HMC. Dodecanoic acid was the most effective in identifying HMC hams, indeed, it increased from 0 to 18th month. The other high-discriminant compounds followed the same trend during ripening, except for 3-octen-2one, whose lower occurrence in HMC than in LMC, acted as the discriminant factor. Secondly, the SDA was applied only to HMC hams (12, 14, 16 and 18 seasoning months), selecting 17 compounds. Samples seasoned for 14 and 18 months resulted separated (P=0.02). CAN analysis also differentiated "12 months" seasoning class from the other classes and "18 months" class from "16 months" one. The main VOCs associated to the "12 months" class were nonanal, 1,5-Diphenyl-3methylthio-1,2,4-triazole and 6-methoxy 2-hexanone. Also 3-nonen-5-yne,4-methyl played an important role in the characterization of 18 months samples respect to 14 and 16 ones. The 26 compounds identified in the first scenario could be a useful tool to determine the ripening status of unknown samples. Despite the loss of accuracy observed in the second scenario, also the 17 compounds identified as discriminating within the HMC samples, turned out to be an interesting way to separate at least 12th months samples from 18th ones.

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ence. Italy ABSTRACT

# Gas chromatography-ion mobility spectrometry: a portable instrument to ensure Iberian ham traceability during its processing

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Dry-cured Iberian ham is a highly appreciated food product, which is obtained from Iberian pigs fattened on grazing acorns after a lengthy time period (>2 months) and several stages of processing (>3 years). Consequently, a fraudulent labelling during any of these stages implies a serious damage to consumers and legitimate producers. Therefore, the main objective of this study was the classification of Iberian hams samples during their processing according to the pig's feeding regime and breed purity using a gas chromatography-ion mobility spectrometry (GC-IMS) device. This instrument allows to perform a qualitative and quantitative analysis if it is necessary. This means that the GC-IMS can be used for target (identification and quantification of features) and untargeted (using all data found in the topographic plot) analysis. Using the same instrument, samples of different nature (lard, meat or fat) can be measured allowing the traceability of the pig production chain from the slaughterhouse to the final product provided to consumers. In this project, more than 80 samples of raw Iberian pig fat, 120 samples of dry-cured ham fat and 81 samples of dry-cured ham slices were analyzed. The raw fat samples were extracted from pig carcasses immediately after slaughtering. Additionally, cured fat and slice samples were extracted from ham pieces during and after their curing process in industrial cellars, respectively. All fat samples were obtained using a novel non-destructive sampling method based on a puncture with needles. The employed instrumental parameters for needle and slice analyses were described in previous works. In this project, these methods were slightly reoptimized to reduce measurement analysis time. The potential of untargeted approach was recently demonstrated analysing dry-cured ham samples. In that approach, the intensity of 85 features found in GC-IMS topographic maps was analysed by chemometric techniques (orthogonal partial least squares discriminant analysis). The results showed high validated discrimination rates (91.7% using cured fat, and 100% using slices) between drycured ham samples of two different categories based on the feeding regimes provided to the Iberian pigs: acorn-fed or feed-fed. On the other hand, discrimination between hams of Iberian purebred and crossbred pigs was of 100% with cured fat and 70% with slices. Raw fat data are currently under study. A target approach is also possible using a GC-IMS device. In this case, qualitative and quantitative analysis are under study. 51 volatile organic compounds (VOCs) previously described as present in Iberian hams were individually analysed, dissolving each one in refined oil (as this matrix is similar to liquid fat) with the aforementioned methods. A mixture of 31 VOCs was identified in cured fat samples (the rest of the compounds were not detected). 20 out of 31 VOCs were simultaneously quantified in one sample. A critical comparison is being carried out between untargeted-target approaches to check which one will be the best to classify samples according to the feeding regime supplied to the Iberian pigs.

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# Fatty acid composition, lipogenic enzyme activities and increased thickness of subcutaneous adipose tissue of two lines of Iberian pigs subjected to three types of feed during the fattening period

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Genetic and feeding are two fundamental factors affecting the Iberian pig production. The objective of the present study was to evaluate the effect of genetic and diet, and their interaction, on adipose tissue composition, growing and metabolism of Iberian pigs during the final fattening period. The study used 48 castrated male Iberian pigs divided in 6 groups (n=8) following a 2×3 factorial design according to genotype (Retinto and Torbiscal) and diet (3 groups of pigs fed with different level of oleic acid enrichment during the fattening phase: Low, Medium and High, with 0.93, 2.28 and 3.79 g of oleic acid per 100 g of concentrate, respectively). Animals were fattened ad libitum from 102.3±6.3 to 151.3±10.3 kg body weight for approximately 60 days. Measurement of backfat depth was recorded at the level of the last rib at the beginning and end of the fattening phase. After slaughter, backfat samples were taken and analyzed for lipogenic enzymes and fatty acid composition. Significance of difference (P < 0.05) between treatments was determined by two-way ANOVA followed by Tukey multiple comparison test. At the beginning of the fattening period Retinto pigs showed lower live weight and higher fatness (p< 0.05) (100.2 kg and 4.64 cm) than Torbiscal ones (104.4 kg and 3.74 cm). Backfat depth increased by 2 cm in both, Retinto and Torbiscal, during the fattening period. Nevertheless, Retinto pigs exhibited lower weight increase than Torbiscal pigs (p<0.05) (45.0 kg and 52.9 kg, respectively). Related to lipogenic enzyme activities, only glucose 6-phosphate dehydrogenase (G6PD) was affected (p<0.05) by Iberian pig line with significant higher activity in Torbiscal compared to Retinto. This difference in G6PD activity did not affect the fatty acid composition of subcutaneous fat (p> 0.05). At the end of the fattening period, pigs fed on Medium diet showed a higher live weight, with also higher average concentrate intake, followed by High and finally Low diet. However, diet did not affect the subcutaneous adipose thickness. Regarding fatty acid composition, the level of oleic acid enrichment of the diet affected both saturated and monounsaturated, with higher level of saturated and lower of monunsaturated in Low, followed by Medium and finally High diet, respectively. Except for malic enzyme, diet significantly affected the lipogenic enzyme activity, being the concentrate intake and total weight gain determined by G6PD, while lipid diet intake is determined by fatty acid synthase (FAS) and glycerol-3-phosphate dehydrogenase (G3PD) enzymes.

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# Effects of an n-3 Polyunsaturated Fatty Acids - Rich Diet on Nitrogen Metabolism in Growing Barrows

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Previously researches highlighted that up to 30% of nitrogen is converted to food and the rest is excreted by animals. Extruded linseed (LE) and walnut meal (WM) are both of them useful resource if we consider additional of an n-3 enrichment diets and lowering of n-6: n-3 PUFA ratio. The aim of this research was to assess changes in nitrogen excreted and nitrogen concentration in different tissues (plasma, heart, spleen, liver and cecum) by dietary addition of LE: WM (50:50). Ten crossbred barrows Topigs,  $81 \pm 3$  d old, 31.5 (SD 1.90) kg initial weight, were randomly assigned for 3 weeks to 2 groups; SM and LEW, each with five replicates. After 7d of adaptation two balance period followed during which faecal matter and urine were collected. A semiautomatic Kjeldahl method (Kjeltec Auto 1030 Analyzer, Hillerod, Denmark) was used for determining the nitrogen content in the excretion. Nitrogen digestibility, balanced or retained, were determined by measuring nitrogen intake and nitrogen excretion (DM basis). Blood samples were collected by jugular venipuncture in heparin tubes, then centrifuged (3000 rpm for 15 min) for plasma separation. A chemistry analyser (Spotchem EZ SP-4430) was used in order to assess nitrogen level and urea nitrogen (PUN). The dietary addition of LE: WM 50:50 (wt/wt) reduced up to 7.81% n-6: n-3 PUFA ratio in our diet. At the end of balance experiment the animals were slaughtered in order to determine nitrogen tissue composition. The nitrogen intake was similar between treatments. A positive nitrogen balance was noticed that indicate the nitrogen intake exceed the nitrogen lost. However, an n-3 rich diet conducted to an increase of nitrogen retained (>8.09%). Biological value of feed protein was 14.8% higher in LEW diet vs. SM diet. We found a decrease by 14.66% of total nitrogen output in LEW fed group compared to SM group (P<0.05). A positive correlation was registered between dietary n-6: n-3 PUFA and urinary nitrogen output (r = 0.78, P<0.001). In plasma we noticed a similar level of nitrogen (89 g/mL). The liver from LEW diet had greater nitrogen concentration whereas in heart and spleen the nitrogen level was lower (P>0.05). Overall, the results show that an n-3 FA enrichment diet had a significant impact on nitrogen metabolism which led to a higher efficiency of nitrogen utilisation. Addition of LE: WM mixture reduced nitrogen excreted and net protein utilisation. More than 40% of nitrogen was retained.

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ABSTRACT

# Effects of millet grain diet on performance and certain plasma parameters of growing pigs

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In a world facing climatically changes conditions, drought and heat tolerance crops represented keystone to avert food shortage and famine. In this context, millet grain (Panicum miliaceum) have a great potential as an alternative source of energy for pigs. A trial was conducted to investigate the effect of millet grain (cv. Marius) on performance and certain plasma parameters (protein profile) in growing pigs. A total of 40 growing pigs (13.58 kg±0.37), 51±3 days of age, were randomly divided to 2 groups: control group (C) with a classical diet (corn-triticale-soybean meal) and millet group (M, where millet replaces 25% triticale). The diets were isocaloric and isoenergetic. The performances (body weight, BW; feed intake, FI; average daily gain, ADG; feed efficiency, FE) were evaluated after 30d. At the beginning and at the end of the experimental period the blood samples were collected from jugular vein (n=10). Spotchem EZ SP-4430 (Arkray, Japan) was used to determine the plasma protein parameters (total protein, T-Pro; albumin, Alb; globulin, Glob; albumin/globulin Ratio, A/G Ratio; creatinine, Cre; total bilirubin, T-Bil; uric acid, UA; urea nitrogen, PUN). Data were analysed using the SPSS V.20 (2011) software, General Linear Model procedure. The effect was considered significant at P<0.05 and as trend at 0.1<P>0.05. The BW (+6.10%) and ADG (+6.25%) of pigs fed M diet were insignificantly increase at 30d compared to C. The plasma protein parameters obtained at the beginning of the experiment was within the reference ranges. The dietary millet manifested a tendency to decrease the plasma PUN concentration (15.77%, P=0.056), whereas the other plasma protein constituents were comparable to C diet and in normal limits. The results of the present study suggest that millet does not significantly improve the performance of growing pigs, while plasma protein parameters as important marker of health and nutritional status range in normal values for this category. Also, decreasing the PUN concentration in peripheral blood could be the result of a higher utilisation of amino acids provided by millet diet. Thus, millet could be included up to 25% in pig diets and considered a good alternative for replacing triticale.

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Performance of growing Iberian pigs fed diets of different Ca and P concentration

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The potential for lean growth of Iberians pig is low compared to conventional pigs. Accordingly, protein requirements along their productive cycle are lower than those for pigs of higher growth potential. This metabolic profile suggests also lower Ca and P requirements. Before the final extensive fattening during montanera Iberian pigs are reared in conditions which allow monitoring the dietary supply of these minerals –with a fundamental role in bone growth- to obtain an optimum skeletal development to face the finishing stage. The aim of this study was to investigate performance and Ca and P metabolism in Iberian pigs fed diets of increasing Ca and P concentrations. Twenty-four pure castrated male Iberian pigs of 45 kg BW were distributed in 4 groups (6 pigs/group) allocated to one of 4 diets of identical nutritional composition (130 g crude protein (CP), 9.2 g total lysine and 12.5 MJ ME per kg), except for their Ca and P contents (3.9 to 6.4 g Ca, and 3.5 to 5.7 g P per kg, respectively, maintaining similar Ca/P ratio; 1.12). Pigs were fed close to ad libitum and individually monitored for weight and intake from 45 to 80 kg BW. At 70 kg BW digestibility and balance experiments were performed to determine Ca and P losses in faeces and urine. Pigs were slaughtered at 80 kg BW. Blood was sampled and carcass measurements determined. There were no differences in intake, growth rate and feed efficiency among dietary treatments (in average, 2766 g DM/day, 704 g/day and 0.25g/g, respectively, P>0.05). Ca and P plasma concentrations did not differ among pig groups (103 and 131 mg/L respectively; P>0.05). No differences were detected for carcass yield, weights of hams, shoulders and loins, backfat thickness, and carcass length (P>0.05). Ca and P dietary contents did not influence digestibility coefficients for dry matter, organic matter and gross energy (0.814, 0.833, 0.799, respectively; P>0.05), and neither affected N digestibility and N retention (0.719 and 0.253, respectively, P>0.05). Average Ca and P intakes differed among treatments (14.6 to 23 g Ca/day, and 10.6 to 17.5 g P/day: P<0.001), according to the experiment design. Ca and P digestibility and retention were not modified by Ca and P intake (P>0.05). P absorption (g/day) decreased as P intake increased (P<0.01). Average Ca and P retention coefficients (0.30 and 0.16, respectively) were considerably lower than in younger pigs, indicating a decrease in metabolic efficiency for retention of ingested Ca and P as pigs get older. Average Ca and P retention were 5.5 and 2.3 g/day, respectively, with no differences among treatments (P>0.05). The present results indicate a low metabolic efficiency of fattening Iberian pig for Ca and P retention and provide a first assessment of Ca and P requirement for premontanera Iberian pigs. Further results will complete the information required to provide adequate estimations for maintenance and growth requirements of Ca and P during premontanera to ensure economic and environmental sustainability in Iberian pig production.

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orence. Italy ABSTRACT

## Effects of Maternal Hydroxytyrosol Supplementation on Fatty Acids Composition of Iberian Swine Fetuses

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Intrauterine growth restriction (IUGR) is the consequence of inadequate placental supply of oxygen and/or nutrients during prenatal development. IUGR is related to a low antioxidant capacity and therefore increased lipid peroxidation at the feto-placental unit. Lipids, mainly essential fatty acids (FA), are crucial for an adequate fetal development. An excessive lipid peroxidation may compromise their availability, exacerbating the IUGR. Hydroxytyrosol is a polyphenol present in olive leaves and fruits, with prominent antioxidant, metabolism-regulatory, anti-inflammatory and immuno-modulatory properties, which have shown promising effects for preventing or alleviating IUGR processes. Fetuses from pregnancies supplemented with hydroxytyrosol have a higher antioxidant capacity, diminishing lipid peroxidation. Hence, in the present study, we aimed to determine whether maternal hydroxytyrosol supplementation may favor FA availability both in muscle and liver which are pivotal for the metabolism of glucose and lipids. The study involved 13 undernourished Iberian sows, 7 of which acted as the control group (C group), whilst the 6 remaining females (HTX group) were treated with 1.5mg of hydroxytyrosol per kg of feed from day 35 to 100 of pregnancy (day of sampling). Data were analyzed using SPSS 22.0 (IBM, NY). Effects of diet (C group vs. HTX group) on fetal fatty-acid composition were assessed by two-way ANOVA and the sow was considered the experimental unit. The analysis of FA composition in 55 fetuses in the C group and 45 fetuses in the HTX group indicated that hydroxytyrosol supplementation induces significant changes in the FA composition of the fetal tissues. Main effects were found in both the neutral (triglycerides) and polar (phospholipids) lipid fractions of the longissimus dorsi muscle, where hydroxytyrosol decreased the amount of saturated FA (SFA) and increased the amounts of polyunsaturated FA (PUFA), omega-3 (Σn-3) and omega-6 FA (Σn-6), the ratio Σn-6/Σn-3 and the desaturation index (UI). In the HTX group, liver also showed a decrease of SFA in both the neutral and polar fractions but, conversely to muscle, there were few other effects on the neutral fraction. Main effects were found at the polar fraction where, similarly to the muscle, hydroxytyrosol increases PUFA, Σn-3, Σn-6 and UI. In conclusion, the data from the present study evidence that maternal hydroxytyrosol supplementation significantly increase the PUFA content of the fetal tissues.

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