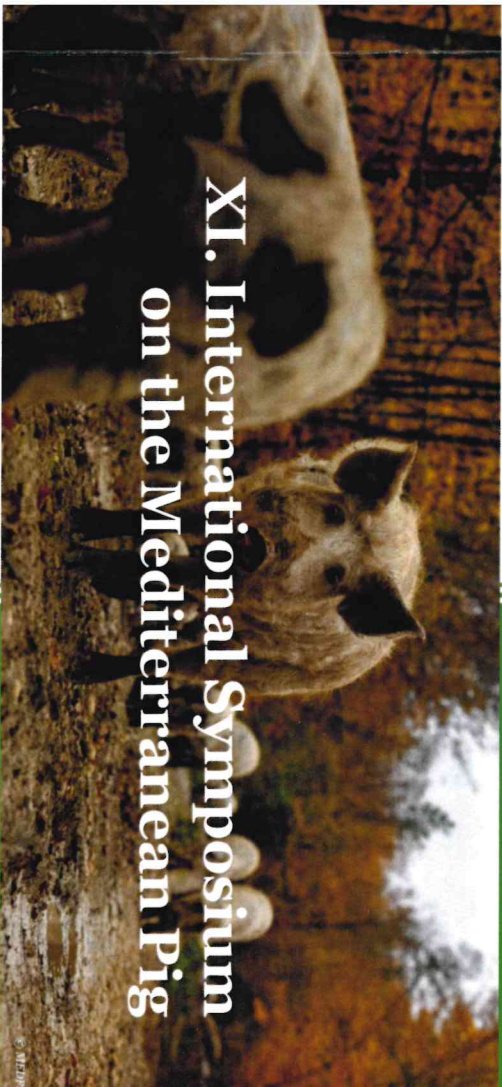


# BOOK OF ABSTRACTS

## XI. International Symposium on the Mediterranean Pig



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

**Keywords:** Pigs, genetic diversity, selection, optimal contributions

## Pedigree verification and parentage assignment using genomic information in Krškopolje pig

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



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

**Keywords:** Krškopolje pig, pedigree verification, genomic data

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## **Can we get rid of autozygosity in the crossbred pig?**

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

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

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

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## Implementation of traceability in Krškopolje pig

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



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

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

#### Acknowledgement

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

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

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Zdravka Vidić<sup>1</sup>, Tomislav Dujčić<sup>1</sup>, Ines Skoko<sup>1</sup>, Anđelo Katić<sup>1</sup>, Ante  
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The aim of this study was to characterize dry-cured hams from four different processing methods, with differences in primary leg treatment, salting and smoking phase. Many biochemical changes (lipolysis, proteolysis, oxidation reactions, Strecker degradation and Maillard reactions) take place during the manufacturing and ripening of dry-cured ham and contribute to flavour development. The samples used in this study were four types of Croatian dry-cured hams of which three with Protected Geographical Indications (PGI) (Krčki, Dalmatinski and Drniški pršut) and one with Protected Designation of Origin (PDO) (Istarski pršut). The research was carried out on 24 dry-cured hams, obtained by processing 24 pig legs following the different specifications. Processing of dry-cured hams was performed according to four protocols, which differ in the stages of smoking (smoked dry-cured ham: Dalmatinski and Drniški pršut) and salting with a mixture of spices (non-smoked dry-cured ham: Istarski and Krčki pršut). Sensory analysis was carried out by a trained panel (8 judges), selected and trained following the procedures of the ISO standard (ISO 11132: 2012). Furthermore, the precision and trueness of each expert assessor were determined by the repeatability index (R<sub>ia</sub>), and intermediate precision index (IPI<sub>ia</sub>), and homogeneity of panel CVR%. The following attributes were evaluated: color intensity of muscle and fat tissue, color uniformity, amount of intramuscular fat (marbling), surface moisture, tyrosine crystal coverage, odor intensity, hardness,

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## Implementation of traceability in Krškopolje pig

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Milena Kovač, Špela Malovrh



## Traceability regulations for pigs

### • LEFT EAR



### • Identification number

- Breeding programme for local Krškopolje pig



### • RIGHT EAR



- Identifying the group of pigs to determine the farm of birth
- Rules on the identification and registration of pigs (EU Directive)

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## Aim of the research



- Implement methods for traceability and verification of pigs and meat with the help of genomic information

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## Materials

Breed	Krškopolje pig	Modern genotypes
Analysing since	2019	2021
Tissue samples	1583	546
Meat samples	43	/
illumina SNPchip	80K	50K
Quality Control	1494 pig samples 27 meat samples 40.002 SNP	530 pig samples / 40.669 SNP

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## Methods

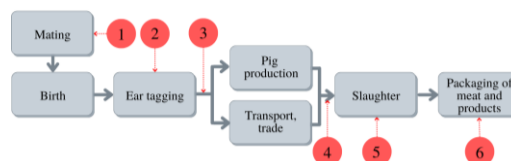
- A** Parentage assignment out of potential sires and dams with known litter
- Each mating of sire and dam must be recorded in the database
- B** Looking for maximum genomic relationship coefficient between meat samples and Krškopolje pig or other modern genotypes
- C** Breed assessment ( **A** & **B** )

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## Results

### Critical points in pig supply chain



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## 1 Critical point – Mating

- Misidentification of boar
- Harem breeding

Zootechnical documentation  
DNEVNIK PRIPUSTOV IN OSEMENTEV / HAREMSKI P.

Datum prijava / osemnitve (S je znak)	Datum vključitve svinje v harem (S je znak)	Učesna številka svinje	Učesna št. merjence	Letniški merjajec (primak lastnika)	Opombe

\* S je za svinjo v haremotam pripravljen in nekaj dni, se zapise datum od \_\_\_\_ do \_\_\_\_  
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## 2 Critical point – Marking

- Ear tagging every live piglet till weaning or mixing
- Possible mistakes:
  - Piglets are mixed
  - Late marking
  - Lost records
  - False marking

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## Critical points

### 3 Loss of ID ear tag



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### 4 Transport and trade

Missing records

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## 5 Critical point – Slaughter



## 6 Critical point – Processing meat



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## Breed assessment of meat

- 6 samples are suspected to be crossed with modern genotypes (MG) in previous generations



Type of meat	A		B		C
	Sire	Dam	KP	MG	Breed assessment
Shoulder-1	✓	✓	0,6780	0,0746	✓
Tunka	✓	✓	0,4809	0,0140	✓
Aged loin	✓	-	0,4798	0,0295	✓
Pork chop	-	-	0,3455	0,0000	*
Neck-1	-	-	0,4624	0,0153	*
Shoulder-2	-	-	0,0827	<b>0,2049</b>	! 55
Shoulder-3	-	-	0,0562	<b>0,1783</b>	! 44
Ham	-	-	0,0555	<b>0,1891</b>	! 11
Neck-1	-	-	<b>0,2088</b>	0,1043	! 54
Fresh meat	-	-	0,1246	<b>0,1662</b>	! 44
Roasted meat	-	-	<b>0,1855</b>	0,1235	! 44

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## Breed assessment of dry meat

Type of meat	A		B		C
	Sire	Dam	KP	MG	Breed assessment
Dry bacon-1	✓	✓	0,5000	0,0000	✓
Dry bacon-2	✓	✓	0,3044	0,0000	✓
Dry bacon-3	✓	✓	0,4701	0,0887	✓
Dry bacon-4	✓	✓	0,3572	0,0000	✓
Dry neck-1	✓	✓	0,5529	0,0000	✓
Dry neck-2	✓	✓	0,4955	0,0362	✓
Dry bacon-5	-	✓	0,5064	0,0000	✓
Dry ham	-	✓	0,5221	0,0000	✓
Prosciutto-1	-	✓	0,5079	0,0561	✓
Prosciutto-2	-	-	0,1643	0,0000	*
Dry neck-3	-	-	<b>0,4453</b>	0,0518	*
Dry bacon-6	-	-	<b>0,1742</b>	<b>0,1448</b>	! 1244

! No mating!



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## Breed assessment of meat products from more than one carcass

Type of meat	A		B		C
	Sire	Dam	Max gR	MG	Breed assessment
Dry sausage-1	✓	✓	0,5971	0,1661	*
Dry sausage-2	-	-	0,3466	0,2887	! 43,11
Salami	-	-	0,1788	0,1603	! 43
Mixed meat	-	-	0,1801	0,2736	-

- Mixed meat is harder to analyze

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## Conclusions

- Identified 6 critical points in supply chain of Krškopolje pig meat
- With parentage assignment and then breed assessment the origin of meat can be confirmed
- The origin (especially fresh meat) was falsified by breeder or producers
- The methods cannot be used for meat from more than one carcass
- Further research on informative SNPs for Krškopolje pig

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Thank you for your attention!



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