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High-tech science for small scale pork producers

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Summary

Today most of the scientific research is aimed at revealing the biological basis underlying the economic traits in pigs which often results in shortcomings due to biological trade-off naturally occurring in all domestic animals. Local (indigenous) pig breeds have not been submitted to selective breeding for productivity and the revived interest for them is also related to their reputation of having an excellent meat quality. However, in terms of scientific evidence, the performances and products of local pig breeds are mainly untapped and market potential of their products unexploited. For that reason, there is a need for research activities in order to reveal the potentials of local pig breeds as the basis for the production of special quality pork and pork products. This review describes advanced scientific tools that are being used for characterisation of local pig breeds and their products within the "TREASURE" project financed as Research and Innovation action under the European programme Horizon 2020 and some of the activities undertaken within a scope of complementary project financed by the Croatian Science Foundation "Scientifically Branded Pork".

Key words: *advanced scientific technology, local pig breeds, family farms, traditional products*

Introduction

We love pigs, but we also love pork. The fact that pig meat represent almost 37% of total world meat production (FAO Food outlook, 2017) leaves no doubt that the interest for pigs is mainly driven by high appreciation of pork as a food for human consumption. Indeed, pig meat was the most consumed meat in 2016 with the share of 39.8% in total world meat consumption (USDA, 2016). The consumption of pig meat in EU extended to 32.4 kg per capita in 2015, almost 1 kg more than in 2014. The production of pig meat in 2016 reached 23 million tons, three times as high in comparison with bovine meat, positioning pork as principal meat in EU-28 countries representing 9% of its total agricultural output (Eurostat, 2016). In 2016, over 257 million pigs were slaughtered in the European Union, accounting for 2 million more than in 2015 and 5.2 million more than 10 years ago (Eurostat, 2017).

Such production could not be achieved without highly effective support of scientific research on the important issues concerning animal health, nutrition, genetics and production management. The scientific effort over the years resulted in improved animals with superior reproductive, fattening and carcass traits. These pigs are produced on modern, industrial farms that can assure ideal environment for the expression of their genetic potential. This is perhaps the main reason why three quarters of pig production in the EU is reared by 1.5% of the biggest pig producing companies, leaving small scale producers a marginal role. In this context, the size became a vital constituent of the economic effectiveness of pig farms. It can be noted that smaller farms are also hindered by their technical inefficiencies whereas the large farms achieve better performance benefiting from both, better technical efficiency and superior economy of scale (Pig farming sector - statistical portrait 2014). Increased technical efficiency means tight connection with science.

The big part of the growing body of knowledge and innovative ideas coming from the scientific research are aimed at the revealing of biological basis underlying the important economic traits in pigs and at the involvement of novel technologies in phenotyping of the animals, both in vivo and post mortem. The prerequisite of such development was the expansion of genomics and genomic selection in the most relevant livestock species. The main advances that enabled the implementation of genomic research in domestic animals were the sequencing of their genome, the development of high throughput genotyping technologies and the development of statistical methods to estimate the allelic effects of thousands of markers in data sets of limited number of animals (Samore and Fontanesi, 2016).

However, the bulk of biological knowledge applied in the research of livestock is nowadays integrated under unique term ‘omics’. As explained by Te Pas et al. (2017), ‘omics’ technologies are applied in pig research with the aim to measure the expression of pig’s genome at all levels: DNA (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics). This allows for adopting the holistic view of the molecules that make up a specific cell or tissue and their association with the phenotype of interest which is the first step in the development of biomarkers. Biomarkers can be viewed as a molecular basis of biological processes that regulate the phenotype of an animal (Te Pas et al., 2017). The basic idea employed here is that the mere use of genetic markers is not sufficient for the prediction of the trait of interest due to the interactions with environment. The solution to this problem is the usage of biomarkers that take into account environmental influences by uncovering the expression level of genes, or abundance of proteins or metabolites associated with certain phenotype. Good example for this is the application of ‘omics’ technologies for meat quality management in pork where meat quality was treated as a rather complex phenotype and number of biomarkers were identified and validated in France (Damon et al., 2013).

Regarding the phenotyping methods, a number of non-destructive or non-invasive methods evolved which can accurately assess the body composition of domestic animals. Most widely used in the investigation on pigs are computer aided tomography (CT), dual energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) (Scholz et al., 2016). These methods can be applied on the living animals which is especially interesting when investigating the growth of pigs (Kusec et al., 2016). However, other applications of these biophysical methods are also known. For example the usage of MRI for the texture analyses of meat and meat products (Bajd et al., 2017) or near infrared spectroscopy (NIRS) that can be used for number of analyses on fresh, but also on processed meat (Prieto et al., 2009), where it can replace conventional chemistry methods if the calibration was thoroughly performed (Font i Furnols et al., 2015).

The application of such sophisticated methods demands the development of statistical and mathematical approaches such as genomic best linear unbiased prediction (GBLUP) or a number of Bayesian methods in genetic research (Ball, 2013). Big data analysis, data mining, machine learning and other computational disciplines are nowadays commonly applied in the aim of deciphering messages hidden in the cells of animals of interest. Mathematical modelling used in animal science today ranges from different growth functions like

Richards, Gompertz and von Beratallanfy, used to describe the growth of pigs (Luo et al., 2015), to the theory of deterministic chaos and the use of fractal dimensions in the description of ham slices (Mendoza et al., 2009).

The production of first hamburger grown in the laboratory from a stem cells in 2013 leaves no doubt that the future of meat production is most certainly in the hands of the science. The question is, if this technology continues to develop to affect the social, cultural, environmental and economic issues, how the society as a whole can prepare for such changes (Matick and Allenby, 2013).

It is fair to say that today the most advanced tools are used on a daily basis for the improvement of economically important traits of pigs. Having this in mind it is not surprising that pig industry disposes with genetically improved, productive pig breeds on a global scale and such successful production of pork. On the other hand, the question is where does this situation leave small scale pig producers? Their chances may lay in the fact that the pigs improved by such high-tech research actually have certain shortcomings resulting from biological trade-off that naturally occurs in all domesticated animals. For example, high capacity in the production of progeny may have resulted in lower resilience of sows, or super-muscularity of genetically improved pigs could be responsible for the lower quality of their products. In addition, small family owned farms can count on the existence of niche market for traditional pork products that need some extra qualities which cannot be delivered by usual, commercial type of pigs. Local breeds, on the other hand, although being less productive, bring the desired meat quality but also a sort of brand, tradition, regional identity to the product and thus the added value that can help small scale pig producers to survive. Furthermore, animal welfare is an issue of growing concern for European consumers (Blokhuis et al., 2008; Napolitano et al., 2010). Although there is still a gap between consumers positive attitudes toward animal welfare and their actual buying choices (European Commission, 2007), the incorporation of information about the farming conditions and implied animal welfare as a product extrinsic cues is likely to become one of the key issues in orienting consumer preference. In particular this could be relevant for marketing of meat and meat products from local pig breeds, often reared in traditional small scale production systems (typically low input outdoor systems) linked to a specific environment (Bozzi and Crovetti, 2013), since outdoor pig production, in general, has a favourable consumer perception of perceived attributes of quality, i.e. consumers consider it as more humane, environmentally friendly, traditional and sustainable, and they often have a positive attitudes toward the wholesomeness of meat from such production (Edwards, 2005).

TREASURE project

Čandek Potokar et al. (2015) argued that in terms of scientific substantiation, the performances and products of local pig breeds are practically untapped and market potential of their products unexploited. For that reason, there is a need for research activities in order to reveal the prospects of local pig breeds as the basis for the production of special quality pork and pork products. Exactly this is the key point of the on-going project titled “Diversity of local pig breeds and production systems for high quality traditional products and sustainable pork chains (TREASURE)”.

The TREASURE project is financed as Research and Innovation action under the European programme Horizon 2020 (grant agreement 634476). The consortium of 25 partners from 9 countries has been granted in the frame of the call “Traditional resource for agricultural diversity and the food chain (SFS-07a-2014)”. The project is based on the holistic approach in an attempt of covering all of the important aspects of pork chain sustainability, starting from feeding resources, nutritional requirements, inherent genetic potential of local pig breeds, innovative feeding and management strategies, product fabrication and evaluation till product marketing. These objectives certainly need the involvement of the state of the art scientific knowledge and equipment, starting from genomic laboratories to the sophisticated devices for the investigations of chemical composition of fresh pork and pork products as well as for studying carcass composition of local pig breeds, just as it is used in highly selected pig breeds, reared at large industrial farms.

Within a scope of the present review, a short description of genetic/genomic studies will be presented as well as ongoing investigations on biomarkers for meat quality, application of NIRS technology and CT carried out on local pig breeds and products studied in the frame of the “TREASURE” project.

Genetic/genomic research

Genetics and genomics research can provide useful information for local pig breeds, from both scientific and productive perspectives. In a first place the recently available high throughput tools for the analysis of the genome allow a deep genetic characterisation of the breeds, including the evaluation of their level of polymorphism and genetic diversity, population structure and admixture, or the relationships among breeds and estimation of their genetic distances (Ramos et al., 2009; Ai et al., 2013). For this objective different commercial devices (SNP chips) are available and currently being used in different livestock species. For pigs different commercial SNP chips that exist include from 60K to 600K polymorphisms, and their cost has dropped down in the last years making its use widely accessible. “TREASURE” project includes the genotyping of a relevant number of animals from each one of 20 autochthonous European pig breeds with a high density commercial genotyping platform in order to deeply characterize their genetic singularity and the potential genetic connection between breeds. Data will probably lead us to the identification of genome regions related to between-breed differentiation, or connected to selective sweeps or signatures of demographic events specific of these breeds (Stainton et al., 2017). However, the available commercial chips include neutral markers discovered in modern pig breeds. For this reason the project covers also the study of allele frequency distribution of known major genes (causative polymorphisms) such as genes regulating growth and appetite (e.g. IGF2, MC4R, LEPR), meat quality (e.g. RYR1, PRKAG3, CAST) or coat colour (e.g. MC1R, KIT) which are particularly interesting for breed characterisation or for its potential use in selection schemes. Some preliminary results are already available (Óvilo et al., 2017).

Besides the genotyping platforms, large methodological advances and cost reductions have been also achieved in the massive genome sequencing methods, allowing the employment of such approaches in all scientific scenarios (Esteve-Codina et al., 2013). Massive sequencing of genomic DNA is currently being employed in the frame of the “TREASURE” project for the identification of genome regions or genes responsible of adaptive traits and resilience through the comparison of genomes of our local, well adapted breeds with the ones of modern commercial breeds. Also, the discovery of key genes underlying the biological processes responsible of the distinctness traits in local pig populations will focus on RNA sequencing technology (RNAseq), as gene expression is the most fundamental level at which the genotype gives rise to the phenotype (Ramayo-Caldas et al., 2012). Several pilot transcriptome studies are planned to analyse the transcriptional effects of nutritional and management factors applied on local pig breeds. At last, sequencing methods are also allowing the characterization of the gut microbial ecosystem (metagenomics) and a pilot study on comparison between breeds in the same rearing system or due to the diet or environmental interventions (Frese et al., 2015) is conducted, with a great scientific interest as the intestinal microbiota is emerging as a fundamental factor in host’s health and homeostasis (Xiao et al., 2016).

From the practical perspective, all the foreseen genetic studies will provide tools for authentication, traceability, conservation and breeding programmes. These tools may include the knowledge of the presence of potential favourable or negative alleles in major genes, the development of reasonably priced marker panels (DNA) to improve specific traits, or the identification of functional biomarkers (RNAm) or microbiota “footprints” characteristic for breed/feeding/management groups, with diagnostic usefulness. In short, all the advances contributing to better knowledge and better utilisation and promotion of local genetic resources.

Biomarkers

Pork quality is a complex phenotype determined by interactions between pig genotype and environment. Many factors influencing meat quality (MQ) especially sensory and technological traits have been described, however MQ is still highly variable and difficult to predict at slaughter stage. The identification of biomarkers of MQ and the development of control tools would be helpful for the management of product quality in both conventional and local/alternative pork chains.

Identification of MQ biomarkers has been carried out by transcriptomic approaches on both loin and ham, to reveal genes whose expression level is associated with a quality trait. First studies focused on few MQ traits in differential experimental setup (contrasted groups for intramuscular fat (IMF) content, “destructuration” defect of ham muscles, and shear force of cooled meat, etc.). Biomarkers of pork toughness and of ham destructuration have thus been identified (review of Picard et al., 2015). Regarding IMF that is associated with pork tenderness and juiciness, its variation has been associated to expression level of genes from various functional categories including genes involved in the regulation of adipogenesis during animal growth, making it difficult to identify “robust” molecular predictors of this trait measurable at slaughter stage (Picard et al., 2015).

Another approach to identify biomarkers considered a wide variability of many MQ traits within the same animal design. An experimental design inducing a gradual and high variability in technological and sensory qualities of pork (two breeds produced in different farming systems, n=100; Lebret et al., 2015a) was thus used to identify and validate quality biomarkers. A large number of associations (i.e. potential biomarkers) have been identified between transcripts (microarray gene expression of Longissimus muscle taken 30 min after slaughter) and MQ traits (i.e. 140 significant associations for a* (redness) up to nearly 3000 for tenderness), confirmed by qRT-PCR (more suitable for the development of tools) then tested on other animals from the same experiment. Sixty significant correlations ($R^2 \leq 0.46$, $P<0.05$) involving 26 genes and 8 characters: pHu, water loss, lightness, a*, hue angle, IMF, force shear, tenderness were validated. Thus, the variation in expression level of one gene could explain up to 46% of variation of one MQ trait (Damon et al., 2013). An external validation performed on 100 commercial pigs allowed the validation of 19 of these biomarkers ($R^2 \leq 0.24$, $P<0.05$; Lebret et al., 2013). Multiple regression models including between 3 and 5 genes and explaining up to 59% (hue value) of a MQ variability were also established, but their predictive value tested on commercial pigs was moderate ($r \leq 0.48$, $P \leq 0.01$ between predicted and measured values, Lebret et al., 2013). Furthermore, predictive models have been established using linear (regression, PLS sparse) and non-parametric (random forests) statistics on transcriptomic (microarray) data. Thus, biomarkers of individual MQ traits have been identified and validated, but their predictive value should be improved to foresee the development of control tools.

To improve the prediction level of biomarkers, another strategy based on the identification of biomarkers of sensory and technological MQ classes, i.e. low, acceptable or extra pork quality levels, was considered. Final aim is to propose molecular tools to classify carcasses or primary cuts early after slaughter in pork industries, according to their predicted quality level (Lebret et al., 2015b). Combining scientific expertise and statistical approaches, pork quality classes discriminating both sensory and technological dimensions of pork have been determined using experimental data described above (n=100 pigs). Then, based on gene expression levels in Longissimus muscle taken 30 min p.m., a multinomial generalized model to predict quality class of a given pork sample has been calculated. The model includes 12 genes and allows high accurate classification at cross-validation step, with especially high accuracy for classification of low (88%) and extra (82%) quality samples (Lebret et al., 2015b). External validation will be undertaken within TREASURE project using samples from 4 European local pig breeds (Gascon, Iberian, Krškopolje, Turopolje; Lebret et al., 2017).

Near infrared spectroscopy (NIRS)

Recent years have seen a growing attention by consumers towards diet concerns and the new concept of product quality. Traditional methods to guarantee the wholesomeness and quality of the product sold have however proved to be very expensive over the years, both in economic terms and in terms of time; a series of alternative methods have been developed with the aim of obtaining equally reliable results, but cutting down time and expenses and with the possibility to be integrated into the production process allowing a continuous monitoring of the quality parameters.

NIR (Near Infrared Reflectance) methodology is a fast, non-destructive, non-invasive method that does not require sample preparation and has excellent correlation with the results obtained with 'traditional' chemical analyses. The technique requires a calibration phase on a representative number of samples and a statistical analysis between the chemical data and the properties of the absorption spectra; once the equipment has been calibrated, the accuracy of the estimate is very high. The spectral region appears to be very rich in information: the more significant absorption bands are attributed to the overtones or to the vibrational transaction combinations of bonds related to the functional groups C-H, N-H, OH, namely with the absorption of XHn functional groups. All covalent bonds have absorption bands in the NIR region, while minerals can only be detected in organic complexes and chelates, or for their effects on hydrogen bonds. The NIRS technique, therefore, allows to perform a quantitative analysis for the determination of components that contain these bonds (water, proteins, lipids, carbohydrates) obtaining a characteristic spectrum of the sample. However, the NIRS absorption bands are very large and often overlapped and the use of chemometrics (i.e. mathematical applications to analytical chemistry) is required. The use of NIR spectroscopy for the determination of the chemical composition of food requires thus a calibration phase that involves the identification of samples representations and a statistical study between the chemical composition data and the properties of the spectra. After some mathematical modelling and validation operations, realized with chemometric methods, the instrument can predict the chemical composition of unknown samples with a margin of error defined by the statistical precision of the regression. The methodology has been already used in different livestock species (Barlocco et al., 2006; Prevolnik et al., 2004; Prieto et al., 2006, 2008, 2009; Cecchinato et al., 2011, 2012) and a recent review (Prieto et al., 2017) provided an insight on the strengths and weaknesses of the method in estimating meat quality traits.

In the framework of the TREASURE, NIRS methodology is used to estimate fatty acid composition (FA) of fat tissues (IMF and subcutaneous) as well as IMF of meat and lipid content (LC) of subcutaneous fat. Samples come from 17 different local pig genotypes belonging to 8 different European countries for a total of 552 animals. Additionally, prediction of chemical traits (water, protein, IMF, salt) of pork and products and quality (rheological) traits of dry-cured hams will be tested using NIRS. Analytical data will be coupled with the results of the NIRS analysis in order to obtain prediction equations to estimate the traits considered with rapid and less costly methods.

Computed tomography (CT)

Computed tomography is commonly used in medicine mainly for diagnosis purposes. However, this technology has also been used to evaluate live pigs to develop growth curves (Carabús et al., 2015; Kolstad et al., 1996) and for breeding purposes (Aass et al., 2009), to evaluated carcass (Dobrowolski et al., 2004; Font i Furnols et al., 2009) and cuts composition (Romvári et al., 2005), to determine ossification and articular osteochondrosis (Olstad et al., 2014) or to study salt content (Vestergaard et al., 2004; Fulladosa et al., 2010). Computed tomography is a non-invasive technology based on X-ray that allows differentiating between tissues of different density. X-rays beams are generated, while rotating 360ºC around the object, and after passing through it they reach the detectors. In their way, X-rays have been attenuated in different degree depending on the density of the object they go through, the higher the density, the higher the attenuation. After treatment a reconstruction algorithm a matrix of voxels (3D pixels) is obtained in which the interior part of the object is presented. Each voxel has an attenuation value expressed in Hounsfield units (HU) and it is associated to a colour on a grey scale, from black (less dense) to white (more dense). The volume associated with each HU value allows classifying, by means of segmentation techniques, the tissues into fat, muscle and bone.

Local pig breeds studied at “TREASURE” project are very fatty compared with the commercial white pigs. One of these local breeds is Iberian pig, reared at the Iberian Peninsula. The CT scan images obtained from Iberian carcasses allow to see perfectly this high amount of fat and to take some measurements like fat thickness and fat and muscle area at different anatomical locations. In Figure 1 it is possible to see an example where fat thickness is around 92 mm in the loin region (at 6 cm of the midline and perpendicular to the skin), which is very high compared to the commercial white pigs, that in Spain have an average fat thickness at the same level of around 14 mm. Similarly, when all the images of the carcasses are considered together, it is possible to obtain the lean meat content of the carcass. This is generally less than 30% in pure Iberian pigs, while in commercial white pigs in Spain it is on average 63%.

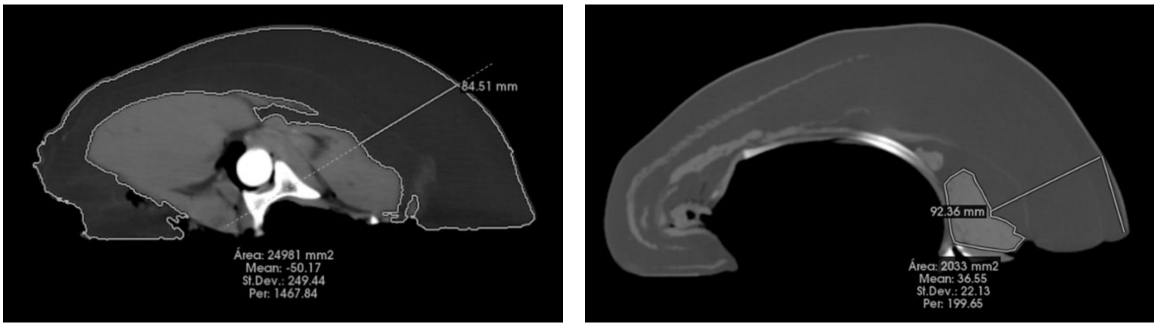

Figure 1: Computed tomography image of ham (left) and loin region (right) of an Iberian pig in grey scale (from white-more dense to black-less dens) and with several measurements of fat thickness and fat and muscle area.

SciBra Pork project

Similar, complementary project is currently running in Croatia, focusing on Crna Slavonska pig breed, titled “Scientifically Branded Pork (SciBra Pork)” under financing of Croatian Science Foundation. As Budimir et al. (2014) explained the objective of this project is to apply various scientific approaches which could help in the creation of new brand among the animal products in Croatia based on Crna Slavonska pigs. Within a scope of this project, the main characteristics of Crna Slavonska breed, such as growth characteristics, physicochemical properties of meat and meat products are undergoing thorough investigations. The results of these can be exploited in the processes of branding of the meat products. For example, the differences in proteolytic and lipolytic activities are found to be a consequence of genetic background combined with the rearing system applied. Considering this it can be assumed that the traditional dry-cured meat products based on the meat of Black Slavonian pigs will have different physical/chemical and sensory characteristics then those produced from meat of commercial pigs. Especially when they are reared outdoors.

Following the above hypothesis, one of the main goals of SciBra Pork project is the identification of typical physicochemical characteristics and sensory profile of traditional dry-cured meat products originated from Black Slavonian pigs. These work include the assessment of fatty acid profile, aroma profile, myoglobin concentration, water activity (aw), pH, colour assessment, free fatty acids quantification (acidity value), extent of lipid (peroxide value; TBARS; volatile aldehyde analysis) and protein oxidation (protein carbonyls quantification). These traits will be compared with the same products originating from different production chains involving commercial pig breeds. Similarly to the TREASURE project, the results of above mentioned research will be at disposal to the small scale producers, some of them involved in the both projects.

Closing remarks

Development of tools for authentication and traceability, conservation and breeding programs through the application of the latest genetic/genomic methods, identification of molecular biomarkers for early prediction and control of meat quality, use of the fast NIRS technology for the assessment of chemical composition of fresh pork and pork products, or investigation of carcass tissue composition by the sophisticated CT devices, may all provide a new, missing data that can bridge the gap in the body of knowledge between industrial and small scale pork production, which is one of the main prerequisites for better utilization of local breeds in the future. Moreover, the results of such investigations could become an excellent base for the branding of products originating from these pigs. After all, we should be able to keep track, understand, comprehend and utilize in most imaginative way, the emerging scientific technology that modern fundamental science can offer for backing up the sustainable production of pork as well as high quality traditional pork products.

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