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# L'ère de la post-génomique en biologie moléculaire : la protéomique

**La post-génomique en biologie moléculaire : l'ère de la protéomique. Applications en sciences animales et en médecine vétérinaire**

**Mots-clés :** Santé Animale, Omics, Médecine vétérinaire, Sciences animales

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**Le second workshop de protéomique organisé par la Faculté de Médecine Vétérinaire de l'Université de Zagreb en Croatie a permis de synthétiser les récentes avancées en protéomique et autres sciences en omics, et leurs applications potentielles en médecine vétérinaire et en sciences animales.**

## Résumé :

L'équipe de la chaire EER (Espace Européen de la Recherche) de Zagreb a été créé dans le cadre du 7<sup>ème</sup> Programme Cadre de Recherche et Développement de l'Union Européenne. Son objective est, pendant quatre ans, d'accroître, d'encourager, de développer et d'élargir les compétences en biologie moléculaire de la Faculté des Sciences Vétérinaires de l'université de Zagreb en Croatie. Différents workshops ont été organisés afin de diffuser les sciences omiques au sein de la médecine vétérinaire. Le dernier workshop, intitulé "L'ère de la post-génomique de la biologie moléculaire - Protéomique ère II", a été organisé à la Faculté de Médecine Vétérinaire de Zagreb du 14 au 16 novembre 2017. Des spécialistes reconnus au plan international ont présenté les avancées et les applications les plus récentes, essentiellement en protéomique mais aussi dans toutes les sciences omiques (génomique, métabolomique, bioinformatique). Les présentations publiées dans cet article illustrent les méthodologies qui sont partagées dans les sciences animales (qualité de la viande, production et bien-être animal) et vétérinaires (santé animale). Les premières présentations décrivent le potentiel de la protéomique et de la bioinformatique en sciences animales et vétérinaires. Ensuite, des exemples pratiques illustrant l'intérêt de la protéomique sont présentés pour différentes espèces (moutons, chèvres, bovins) dans différents domaines (tolérance en perte de poids au cours des saisons, qualité sensorielle de la viande bovine, biomarqueurs invasifs de phénotypes variés, ontologie et phénotypage pour maximiser l'utilisation de la génomique et de la protéomique).

## Abstract: Post-genomic era of molecular biology – Proteomics era II

The ERA (European Research Area) Chair team of Zagreb has been created through the European Union seventh research framework program. The objective is to upgrade, encourage, develop and enlarge the capacities in molecular biology of veterinary medicine at the Faculty of Veterinary Medicine of the University of Zagreb, Croatia, for a 4 years period. Different workshops have been organized to introduce omics science to veterinary medicine. The last one, entitled "Post-genomic era of molecular biology – Proteomics era II", was organized at the Faculty of Veterinary Medicine of Zagreb from 14<sup>th</sup> to 16<sup>th</sup> November 2017. Internationally recognized specialists presented recent advances and applications, mainly in proteomics but including other omics (genomics, metabolomics, bioinformatics). A number of contributions published in this article illustrate shared methodologies between animal sciences (meat quality, animal production and welfare) and veterinary medicine (animal health). The first two contributions described the potential of proteomics and of bioinformatics in animal sciences and veterinary medicine. Then, practical examples of the usefulness of proteomics were described in various species (sheep, goat, cattle) regarding different topics (seasonal weight loss tolerance, eating quality of beef, invasive biomarkers of various phenotypes, ontologies and phenotyping to maximize use of genomics and proteomics).

## INTRODUCTION

The ERA Chair team of Zagreb has been created through the European Union seventh research framework program 2013. Since 2015, the team's aim has been to upgrade, encourage, develop and enlarge the capacities for research in molecular veterinary medicine at the Faculty of Veterinary Medicine of the University of Zagreb, Croatia.

From 14<sup>th</sup> to 16<sup>th</sup> of November 2017, the ERA Chair team organized a workshop entitled "Post-genomic era of molecular biology – Proteomics era II", at the Faculty of Veterinary Medicine of Zagreb. The aim of this workshop was to share knowledge between internationally recognized

specialists in animal sciences and veterinary medicine in the field of molecular biology. As omics is a growing area, it is essential that established methodologies in animal sciences could be adapted to veterinary medicine. Reversely, direct on-field applications of veterinary medicine researches can be adapted to animal sciences. Various speakers were invited from France, Italy, Austria, Canada, United Kingdom and Croatia. Different contributions published in this special issue illustrate shared methodologies between animal sciences (meat quality, animal production and welfare) and veterinary medicine (animal health).

## I. APPLICATION OF GENOMICS AND BIOINFORMATICS TO ANIMAL DISEASE RESEARCH

The European Union is investing in the development of molecular biology research in east and central European countries. Such countries need to increase their omics research potential, as applied to different domains such as animal diseases or meat sciences. Nicolas Guillemin from the Faculty of Veterinary Medicine of the University of Zagreb (Croatia) explained that aims of his team are to develop and improve such omics researches in Croatia through education and project support.

Genomics can be briefly defined as the study of genome composition and expression that leads to phenotypes and traits (Guillemin *et al.*, 2016). There is a need of investment in genomics in countries, which are developing molecular biology research, especially concerning interaction of genomics with proteomics. Those two omics domains are complementary for deciphering traits or diseases. It is so important to establish multi-disciplinary teams and projects.

Bioinformatics can be briefly defined as the collection of *in silico* tools and methods to manage, treat and use data to

generate knowledge (Guillemin *et al.*, 2016). As omics experiments generate more and more data, it becomes crucial to have tools able to manage all this data influx and to generate understandable knowledge from raw data. Such activities lead to hypothetical models, prospection for new biomarkers, therapies or projects.

Bioinformatics exploration of proteomics data related with parasitic infection in dogs highlighted the potential role of lipid peroxidation and oxidative stress management during the infection process, with a hypothetical model constructed from the *in silico* approach.

Such methods represent valuable tools for researches in animal sciences, particularly for countries, which are developing their molecular biology researches. In Croatia, support in omics should continue to improve animal sciences (production, diseases), notably for production of milk and meat from local breeds.

## II. ANIMAL PROTEOMICS: TODAY & TOMORROW

As presented by Ingrid Miller from the University of Veterinary Medicine in Vienna (Austria) and the different speakers, animal proteomics has gained importance during the last years, especially in the field of farm animals (Eckersall, de Almeida, Miller, 2012). There is an increased trend towards studying different aspects of animal health and disease, as well as animal products, most often by modern high-resolution mass spectrometric approaches. While this trend can only be welcomed, resulting in more complete (or corrected) databases and increased knowledge in the field, some caveats need consideration. For instance, pathway analysis, though helpful to find out about protein functions and interactions, is based on existing literature and databases (which may be incomplete or faulty for non-model organisms). Validation of proteomic findings needs to be performed with independent methods, ideally based on additional protein properties (activity testing, antibodies). Systems biology approaches, i.e. data on the same set of samples/animals, help to get a more complete picture and to

confirm proteomic findings. Newly developed methods and algorithms should be critically tested for weak points and artefacts, like any other analytical methods.

Experiences from recent years have pointed out the importance of protein modifications (isoforms, PTMs, size differences), and this concept of proteoforms or protein species will much better explain altered occurrence and processing of molecules in the body. Such investigations stress the importance of top-down analysis, particularly classical gel-based methods (intact protein MS is just in development). Examples show the complementarity of different proteomic approaches (Jungblut, Thiede, Schlüter, 2016). Present-day proteomic methods are now sensitive enough not only to determine biological variation between individuals of a given group, but also to detect sex-dependent differences in the proteomes of non-reproductive tissues (Gianazza, Eberini, Miller, 2018). This aspect calls for additional awareness and consideration in future studies, but may sharpen the conclusions drawn from the investigations.

### III. USING OMICS TO UNDERSTAND SEASONAL WEIGHT LOSS TOLERANCE IN GOATS AND SHEEP

Two examples of genomic application related to seasonal weight loss in goats and sheep were presented by André M. de Almeida from the University of Lisbon (Portugal).

Seasonal weight loss (SWL) is a pressing issue in animal production. Animals selected in SWL prone areas are well adapted to SWL. Understanding the molecular mechanisms of SWL adaptation is of high importance in animal selection. We studied the effect of SWL, on the mammary gland secretory tissue proteome and metabolome in two goat breeds from the Canary Islands with different levels of tolerance to SWL: Majorera (tolerant) and Palmera (susceptible). Within each breed, goats with the same age and stage of lactation were divided into two groups ( $n = 5$ ): control (constant weight) and underfed (15% liveweight reduction). At day 22, mammary gland biopsies were extracted and proteomics and metabolomics profiles obtained.

Protein extracts were obtained and trypsin digested using the FASP protocol. Peptides were loaded onto reverse-phase C18 columns and analyzed on an LTQ-Orbitrap Velos mass spectrometer. Protein identification and label free quantification were performed using Mascot and Progenesis software. Metabolomics: Aqueous fractions were obtained by tissue aqueous/organic extraction. <sup>1</sup>H NMR spectra were collected from the aqueous extract of the mammary gland using an 800 MHz Bruker AvanceII+ spectrometer. Regarding the proteomics component, 1010 proteins were identified, from which 96 were considered statistically different among groups.

SWL led to an increase of proteins related to apoptosis and stress processes in both breeds. Moreover, both breeds showed a decrease in the number of proteins related to protein, carbohydrates and fat biosynthesis. When both breeds were compared after SWL, the Majorera breed showed higher expression of immune system related proteins compared to the Palmera breed. In contrast, the Palmera breed showed higher expression of proteins related to apoptosis, ketone bodies formation (fat liver) and protein metabolic processes compared to the Majorera breed.

Regarding the Metabolomics experiments, we were able to identify 47 different compounds in the aqueous fraction of mammary gland extracts. Lactose, glutamate, glycine, lactate and glucose were found to be the most abundant. Statistical evaluation using Principal Component Analysis (PCA) and Partial Least Squares (PLS) revealed differences between control and underfed animals, although no differences between breeds were observed. In conclusion, the two goat breeds have a different metabolism reaction to SWL,

highlighting differences particularly related to the immune system and apoptosis.

Seasonal Weight Loss (SWL) is also an important constraint, limiting animal production in the Tropics and the Mediterranean. As a result, the study of the productive characteristics and the physiological mechanisms by which domestic animal breeds respond to SWL is important to those interested in animal breeding and the improvement thereof. To that end, proteomics has been instrumental in obtaining important information on the mechanisms underlying SWL. The objective of this research was to characterize the production traits (growth, carcass and meat quality) and the differential protein expression in the skeletal muscle of three different breeds of sheep: the Australian Merino, the Dorper and the Damara, each showing different levels of tolerance to weight loss (low, medium and high, respectively).

Per breed, two experimental groups were established using 6 month-old ram lambs ( $n=12$ ). One group was labelled "Growth" and the other labelled "Restricted." After forty-two days of dietary treatment, all animals were euthanized in a commercial abattoir and carcass and meat traits determined. Muscle samples were then taken and proteomics profiles determined using two-dimensional electrophoresis coupled to mass spectrometry (MALDI-TOF/TOF), as well as label free proteomics.

Results point out to important differences between the merino and the South African breeds (Dorper and Damara) regarding carcass and meat quality traits like carcass yield and meat color (darker in the Damara breed). Regarding the 2D proteomics analysis, results also reveal relevant differences between breeds with several proteins that are suggested as putative biomarkers of tolerance to weight loss: Desmin, Troponin T, Phosphoglucosyltransferase and the Histidine Triad nucleotide-binding protein 1. Moreover, we suggest as related to SWL tolerance: S100-A10, Serpin A3-5-like and Catalase. From the label free proteomics experiment, we observe that the more vulnerable to SWL a breed is, the more differential abundance proteins we find. Protein binding was the most frequently altered molecular function identified. We finally suggest six putative markers for restricted nutritional conditions independently of breed: ferritin heavy-chain; immunoglobulin V lambda chain; transgelin; fatty acid synthase; glutathione S-transferase A2; dihydrodiol dehydrogenase 3-like.

This information is finally of relevance to and of possible use in selection programs aiming towards ruminant animal production in regions prone to droughts and weight loss.

### IV. A STRATEGY FOR BIOMARKER RESEARCH IN BEEF QUALITY

The next conference prepared by Brigitte Picard and Muriel Bonnet from INRA (France) gave another example of proteomic application.

The large variability of tenderness is the main problem for the beef sector from breeders and butchers to consumers, while intramuscular fat content is of interest for the nutritional value of meat. So, there is a major issue to predict beef meat qualities of live animals, carcass or cuts.

For several years, various scientific programs aimed to identify proteomic biomarkers for tenderness (Picard and Gagaoua 2017) and deposition of lipids (Taga *et al.*, 2012).

As exemplified for the tenderness, the first step of the applied strategy was the discovery of biomarkers of tenderness by proteomic analysis comparing proteins differentially abundant between two groups of high versus low tenderness from the *Longissimus thoracis* (fast oxido-glycolytic) and *Semitendinosus* (fast glycolytic) muscles. This provided a list of candidate biomarkers.

Then, immunological tests were developed (dot-blot and Reverse Phase Protein Array) and have been used to measure the relative abundance of the candidate biomarkers of tenderness and evaluate their relationships with tenderness



(Guillemin *et al.*, 2009). Multiple regressions based on relative abundances of these proteins were used to propose equations of prediction of tenderness in different types of cattle (young bulls, cows, heifers and steers of several breeds).

The last step in progress is the development of a final tool for the routine evaluation of tenderness and other beef qualities such as intramuscular fat content, pH, colour, juiciness, flavour, on carcass or on alive animal. Moreover,

## V. QUEST FOR LOW INVASIVE BIOMARKERS OF RUMINANT TRAITS

Then, Isabelle Cassar-Malek and collaborators (M Delosière, L Bernard, S Boudon, J Bazile, M Bonnet) from INRA (France) extended the usefulness of invasive biomarkers to various phenotypes.

A challenge for the ruminant sector is to predict and manage complex phenotypic traits related to production efficiency. A key issue is to develop decision support tools that would assess biomarkers of animal efficiency including body composition, tissue development, feed efficiency, robustness and product quality in the living animals or in carcasses.

Previous research efforts have investigated the potential of tissue-derived markers to assess the metabolism, body composition (muscle and adipose depots), and product qualities (dairy and meat). Catalogues of tissue proteins and candidate biomarkers have been generated through a

## VI. ANIMAL TRAIT ONTOLOGY AND PHENOTYPING OF LIVESTOCK: THE NEW CHALLENGES FOR GENOMICS

Finally, Jean-François Hocquette from INRA (France) highlighted the importance of phenotyping for proteomics, and more generally genomics.

Providing phenotypic information, which is accurate, reliable, repeatable and comparable across countries or laboratories is critical to establish functional relationships between animal genotypes and their phenotypes. For instance, the results of genome-wide association studies are hampered by a low repeatability of trait measurement. Consequently, individuals should be genotyped solely for strongly correlated traits for independent measurements (Barendse, 2011). Furthermore, meta-analyses from combined various sources of phenotypic data of multiple origins may be hampered by the variability in the methods of phenotyping (Chriki *et al.*, 2013). In this context, the standardization and accuracy of phenotype measurements are becoming major limiting factors in genomic approaches (Hocquette *et al.*, 2012). This is the reason why ‘in the age of the genotype, phenotype is king’ (Coffee, 2011).

The ‘Animal Trait Ontology of Livestock’ (ATOL) project aims to define precisely the phenotypes of interest for farm animals (<http://www.atol-ontology.com/rb/en/1>) as previously done for rodents, and to make this reference suitable to support database management, semantic analysis and modeling (Le Bail *et al.*, 2014).

The International Committee for Animal Recording (ICAR) is an International Non-Governmental Organisation

bioinformatic analysis showed that the protein biomarkers of tenderness are involved in the following biological functions: muscle glycolytic and oxidative energy metabolism, calcium metabolism, muscular ultrastructure and contraction, oxidative stress, apoptosis and cell protection (Kaspric *et al.*, 2015). Combined with the biostatistic analysis of the interactions between proteins, new highlights about the biological functions involved in beef tenderness can be proposed (Gagaoua *et al.*, 2015).

combination of omics and *in silico* experiments (Cassar-Malek and Picard, 2016, Bernard *et al.*, 2017).

An integrated data mining of transcriptomic and proteomic data was recently published to computationally predict the large-scale secretome of adipose tissues and muscles in ruminants (Bonnet *et al.*, 2016). Methods for milk proteomics are in development (Delosière *et al.*, 2017). Evaluation of the candidate biomarkers and their validation over different ruminant breeds, production types and conditions are required. The next step is to look for biomarkers in the proteome or metabolome of fluids to enable prediction in the living animals.

Altogether, those results will provide knowledge on the cross-talk of tissues involved in the construction of the phenotypic traits linked to cattle production as well as detection/management tools from a blood/milk test.

(INGO) founded in 1951 in Rome (<http://www.icar.org>) composed of 115 Members from 57 countries. ICAR is one of the leading global providers of Guidelines, Standards and Certification for animal identification, animal recording and animal evaluation.

The United Nations Economic Commission for Europe (UNECE) aims to develop agricultural standards by a demand-driven process for international trade. Standards should reflect trading practices, rather than normative ideals. In the area of beef quality, a great amount of consumer data was collected using the Meat Standards Australia (MSA) protocols in different countries by different research groups, which volunteer to share data for mutual benefits (Bonny *et al.*, 2017). Under the auspice of the UNECE structure, a working group led by Poland aims to address issues of language and data management in order to facilitate adoption of standardised beef traits and of standardised consumer testing protocols with collected data comparable and available in a common cloud-based data platform.

In conclusion, as already initiated for plants and laboratory rodents, the collection of phenotypes requires the setting up of a network of phenotyping platforms specialized in farm animal production. All the above initiatives are likely to improve phenotyping and consequently, to maximize the value of genotyping and make best use of genomics in animal science.

## CONCLUSION

Different topics were presented related to the application of molecular biology to meat quality, animal health (both farm and companion animals), production traits and animal welfare. All those themes, despite their different objectives, share research methodologies and also technical issues.

This workshop was the occasion for researchers to exchange their views about the need to have collaborative and integrative approaches between omics technologies (genomics, proteomics, metabolomics, lipidomics, etc...), to identify biomarkers. Researchers also need more and more accurate and consistent databases to work with, especially concerning farm and companion animals, poorly documented

compared with humans and mouse. Annotation and accurate data collection through phenotyping is a growing and challenging area for researchers both in animal sciences and veterinary medicine. As an example, data collection in meat sciences is a model for veterinary medicine.

This workshop was a good opportunity for researchers working in animal sciences and veterinary medicine to meet, share knowledge, and collaborate on common aims and methodologies. Veterinary sciences need to continue improving their use of molecular biology based on the animal sciences model.

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