

Deciphering organic farming impact on pectoralis major muscle proteome of ross 308 chicken: towards the identification of biomarkers of authenticity

Laura Alessandrini ¹, Gianni Sagratini ¹, Renzo Galli ³, Anne Maria Mullen ², Mohammed Gagaoua ²

¹ University of Camerino, Italy, ² Teagasc Food Research Centre, Ireland, ³ Fileni S.p.A., Italy

Objectives: Poultry meat is among the most commonly consumed meats in the world. The physiological and metabolic functions of the animals are influenced by many factors such as the production systems and pre-slaughter stress, with consequent impact on the quality of the chicken meat [1]. Organic meat production systems aim to provide more sustainable products with high levels of quality and safety and are based on high animal welfare standards [2]. To date there has been a paucity of published literature on the application of high-throughput omics methods such as proteomics to further our understanding and characterisation of these production systems and potential impacts on quality. This study aims to understand the impact of an organic farming system, compared to an antibiotic free system, at the level of the proteome of postmortem *Pectoralis major* muscle in the *Ross 308* strain.

Materials and Methods: Twenty *Ross 308* chickens were used in this study, from which half were reared according to the Council Regulation (EC) No 834/2007 on organic production and labelling of organic products and half were from antibiotic-free inside ground farming. All animals were slaughtered under standardised systems, within the one batch and within one hour at Fileni® industry (Cingoli, Italy). *Pectoralis major* muscle (breast) biopsies were taken within 3 h, with randomisation between the left and right sides, and stored at -80°C. For shotgun proteomics, the protocol of Zhu *et al.* [3] was used for total protein extraction and preparation of the protein bands using one-dimensional SDS-PAGE for quantitative proteomics using SWATH-MS (sequential window acquisition of all theoretical fragment ion spectra mass spectrometry) as in Chantada-Vázquez *et al.* [4]. The proteins differentially expressed using ANOVA at a level of 5% were used for bioinformatics using Metascape® platform to investigate the main enriched Gene Ontology (GO) terms as described by Gagaoua *et al.* [5].

Results and Discussion: A total of 659 proteins were identified by SWATH-MS, among which 61 were differentially expressed proteins (DEPs at $P < 0.05$) between the organic and antibiotic-free farming systems. Of these, 24 DEPs were up-regulated and 37 were down-regulated in organic farming system. Principal component analysis and heatmap clustering of the DEPs allowed a clear separation of the two farming systems. The pathway enrichment analysis of the 61 DEPs identified 20 GO terms to be significantly enriched. The carbon metabolism (has01200) and the pyruvate metabolic process (GO0006090) were the top enriched terms followed by others related to muscle system process, actin binding, structural constituent of cytoskeleton, amino acid metabolic process, and cardiac muscle contraction. Six enriched terms were down-regulated in organic farming and 9 were up-regulated. For the former, most of the terms were linked to structural constituent of cytoskeleton and cellular modified amino acid metabolic process. The up-regulated terms were related to proteins playing a role in binding-properties, ATP-dependent activity, and regulation of important pathways such as cellular catabolic and protein modification processes.

Conclusions: This is the first study using proteomics to investigate the impact of production system on chicken muscle proteome. The organic farming system was found to influence the muscle proteome, compared to the antibiotic-free one. Proteins that exhibited abundance differences can be used as biomarkers of authenticity.

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