Mule duck “foie gras” show different metabolic states according to their quality phenotypes by using a proteomic approach. Comparison of 2 statistical methods

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Yoannah François,[†,‡,#,§] Christel Marie-Etancelin,[‡,#,§] Alain Vignal,[‡,#,§] Didier Viala,[Δ] Stéphane Davail,[†] Caroline Molette[*,#,‡,§]

[†]Université de Pau et des Pays de l'Adour, UMR5254, 40 004 Mont de Marsan Cedex, France
[‡]INRA, UMR1388 Génétique, Physiologie et Systèmes d'Elevage (GenPhySE), 31326 Castanet-Tolosan, France
[#]Université de Toulouse, INPT ENSAT, UMR1388 GenPhySE, 31326 Castanet-Tolosan, France
[§]Université de Toulouse, INPT ENVT, UMR1388 GenPhySE, 31076 Toulouse, France
[Δ]INRA, U0370 PFEMcp, Theix, 63122 Saint Genès Champanelle, France
[#]Corresponding author: Tel: +33 (0)5 34 32 39 06. E-mail: molette@ensat.fr

Introduction

France is the main producer of «foie gras» (72% of world production) and 97 % of this production comes from ducks. The "foie gras" is rich in lipids and as this high fat content is responsible for its organoleptic qualities, too much fat loss during the cooking process is a major problem. Recently, Théron et al. (2011) used a proteomics approach to explore the influence of protein composition on fat loss variability, for a limited range of liver weight and of liver lipid contents. Finally, Kileh-Wais et al. (2013) identified QTL related to several "foie gras" quality traits such as liver weight, lipid rate, protein rate, melting rate, color lightness, collagen rate, although the genes underlying these QTL still have to be discovered.

In this context, the aim of the present study is to decipher mechanisms involved in the quality of "foie gras" by means of a proteomics approach using 2D gel electrophoresis. To deepen our understanding of the mechanism underlying "foie gras" quality variability, complex traits are measured, namely the proportion of protein content in crude liver (crude liver protein content) and in dry liver (dry liver protein content) in addition to the classical liver weight and melting rate traits. The proteomics approach allowed the identification of proteins present at different levels of abundance in the samples studied, in relation to the melting rate level alone or to the other traits by using 2 different statistical procedures. Thereafter, biological pathway analysis gives some information on the metabolic state of the livers in relation to their phenotypes and allows us to better understand the biological mechanisms underlying the variations of "foie gras" quality.

Material and methods

The animals are male mule ducks (n=294). They were bred, over-fed and slaughtered at the experimental unit dedicated to waterfowl (UEPFG, Benquet, Landes).

The 2DE analysis (n=294) was performed according to Théron et al. (2011). Briefly, the proteins were solubilized in a standard buffer (Tris HCl 1.5 M pH 8). Bradford method was used for the protein assay. After an isoelectric focusing (pH range 5-8), the strip is deposited on the surface of SDS-PAGE 12% acrylamide. The gels are stained with brilliant blue G250 and scanned using the scanner ImageScanner III. The image analysis software was performed with Samespots software® (TotalLab Ltd, Newcastle-upon-Tyne, UK). The raw data were first corrected for the fixed effects: the zootechnical ones (crammers and hatching batching effects) and the technical ones (gels quality and batches of each dimension electrophoresis). Then, the proteomic data were analysed according to the 4 quality traits, either using a variance analysis (GLM procedure of SAS®) with each quality trait split in 4 quartiles, or

using a linear regression (REG procedure of SAS®). In both cases, the p-values were corrected for multiple tests with the Benjamini-Hocheberg procedure.

The spots of interest, with different abundances according to the levels of quality parameters (melting rate, protein content and liver weight), were identified by mass spectrometry in proteomics platform of INRA Theix. For Nano-LC-MS/MS Analysis, peptides mixtures were analyzed by online nanoflow liquid chromatography using the Ultimate 3000 RSLC (Dionex, Voisins le Bretonneux, France). For raw data processing, Thermo Proteome Discoverer 1.4 (v:1.4.0.288) was used with MASCOT (v:2.3) for database search (http://www.matrixscience.com). UniP_tax_Aves (150722 sequences) was used as database for protein identification.

Results and discussion

Quartiles of the 3 phenotypic traits are represented in table 1.

Table 1: Repartition in quartiles of phenotypic values.

	Quartile 1	Quartile 2	Quartile 3	Quartile 4
LW (g)	394 ± 75	531 ± 27	621 ± 25	731 ± 59
MR (%)	18,7 ± 5,1	34,8 ± 5	46,5 ± 2,5	55,2 ± 3,9
LprotCc (%)	6,2 ± 0,4	7,1 ± 0,2	7,9 ± 0,2	9,4 ± 1,0
LprotDc (%)	9,0 ± 0,7	10,7 ± 0,4	12,4 ± 0,5	16,3 ± 2,9

LW=Liver Weight, MR=Melting Rate expressed as a percentage of the liver quantity before cooking, LprotCc = Crude Liver protein content expressed as a percentage of the fresh liver weight, LprotDc=Dry liver protein content expressed as a percentage of the dry liver weight.

Whatever the quality trait, 45 spots (30 identified) were found to be differentially expressed by the regression (REG) procedure whereas 35 (23 identified) spots were pointed out with the GLM procedure. Among the 30 identified spots with the REG method, 16 are significantly affected by only one out of the 4 quality traits, 5 are significantly affected by 2 or 3 traits and 9 spots (ALB, FKBP4, ENO1, ANXA5 FASN, PRDX6 and VCP) are common to the 4 traits. Among the 23 protein spots pointed out by the ANOVA, 15 had a variation in abundance following a single trait only (LW, MR or LprotDc), whereas the 7 remaining spots were differentially present following 2 traits simultaneously, only 1 spot (FASN) following 3 traits and none are common to the 4 traits. Among the 23 and 30 spots differentially expressed and identified according to the GLM and REG procedures respectively, 20 are common to both methods, 4 specific to the GLM and 10 to the REG. Whatever the statistical analysis used, the proteins showing variation in our dataset represent several biological pathways such as glycolysis, lipid metabolism, oxidative stress, transport, catabolism and anabolism processes.

The comparison of two approaches ANOVA and REG reveal specific proteins. For example, two heat shock proteins (HSPD1 and HSPA5) disappear when using REG procedure because they did not evolve linearly with the trait of interest (HSP expression in the last quartile with MR is much lower than expected under the linear hypothesis). HSPs are synthesized proteins following stress (Heat Shock Factor) in order to respond to it (Niforou et al., 2014). HSPA5 is of the family of HSP70, a cytosolic protein can translocate into the nucleus during a "heat shock." HSPD1 of the HSP60 family is a mitochondrial chaperone protein that helps for the conformation of proteins imported into the mitochondria. Stimulation of HSP is successive to stress; their non-linearity with respect to the MR suggests that the stimulation is due to a threshold effect and therefore is probably more timely than gradual. Furthermore, their synthesis may be just the time to stimulate and support the synthesis of other overexpressed proteins of cytoprotection in the last quartile of the weight of liver (HYOU1) or the MR (PRDX6). Four proteins were only identified using REG procedure: PARK7 and ANXA2, affected by LW are cytoprotecting proteins such as PRDX6 and HYOU1, PFN2, affected by

MR, is implicated in cell motility and TCP1, affected by LprotDc, is a chaperonin protein. These proteins bring precisions on a link between pathways and phenotypes. Under linear hypothesis, a regression seems more efficient than variance analysis in identifying such links.

When looking at the protein profiles, whose variation in protein abundance levels concerns 2 or more traits, a remarkable opposition appeared between the trend of proteins expression with LW and MR levels on the one hand and LprotDc level on the other hand. If protein expression levels increased with LW (FKBP4, C11orf54, PRDX6, ENO1), it also increased with MR or decreased with LprotDc. Inversely, if protein expression levels decreased with MR (APOA1, ALB and FASN), they increased for LprotDc, with the notable exception of VCP. Proteins which expressions decrease with MR or LW and/or increase with LprotDc represent pathways such as synthesis process (LOC100545435, FASN), anabolism process (DLAT, ME1), transcription regulation (PHB) and transport (APOA1, ALB). On the contrary, proteins which expressions increase with MR or LW and/or decrease with LprotDc represent biological pathways such as response to stress (HYOU, HSPA5, HSPD1, and PRDX6), transport (FABP1), glycolysis (ENO1), and catabolism process.

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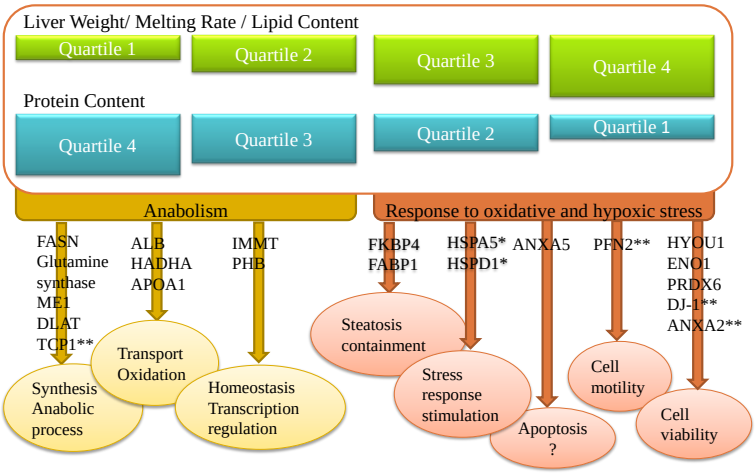

Figure 1: Summary diagram of the different liver protein profiles (from A to F) according to their phenotype. *FASN=Fatty acid synthase, ME1=Malic enzyme, DLAT=Dihydrolipoamide S-acetyltransferase, ALB=Albumin, HADHA=Trifunctional enzyme subunit alpha, APOA1=Apolipoprotein A1, IMMT=Mitochondrial inner membrane protein-like, PHB=Prohibitin, PRDX6=Peroxiredoxin 6, HSPA5=70kDa Heat shock protein 5, HSPD1=60 kDa Heat shock protein, FKBP4=FK506 –Binding Protein 4, FABP1=Fatty acid binding protein 1, ANXA5=Annexin A5, HYOU1=Hypoxia up-regulated protein 1, ENO1=α-enolase, TCP1: T-complex protein 1; PFN2: Profilin 2; ANXA2: Annexin A2; DJ-1 : PARK7*
Protein names with stars were only identified by GLM/ANOVA (1 star) or by REG (2 stars)