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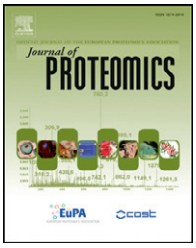

Review

# Current advances in proteomic analysis of (fatty) liver☆

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ABSTRACT

In this review, an overview on proteomic studies conducted in livers of farm animals is conducted with a special focus on liver steatosis in waterfowl. Several studies had interest in understanding liver metabolism in dairy cows under various conditions (e.g. fasting) or the evolution of liver proteome during embryonic phases or growing periods in chicken. Those studies provide interesting results leading to a better understanding of the liver metabolism. Liver steatosis development in waterfowl represents a special case and a focus on proteomic studies conducted in these birds will be done. Indeed, recent studies aimed at resolving protein evolution during overfeeding in duck. Proteomic analysis combining two complementary approaches (2-dimensional electrophoresis gels and shot gun strategy) in order to better understand the mechanisms underlying the variability of cooking yield of fatty liver will be presented.
This article is part of a Special Issue entitled: Farm animal proteomics.



## Contents



## 1. Introduction

Proteomic studies are more and more popular and are used on several animal tissues. The most common method remains to be the two-dimensional electrophoresis (2-DE) gels to separate extracted proteins, and mass spectrometry (MS) to identify proteins of interest. However, other tools are also available such as the liquid-chromatography coupled to MS,

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protein chips, protein/protein complexes in solution or shotgun approaches. Since the years 2000, proteomics developed in animal sciences. Two different strategies can be followed when using these proteomic tools (2-DE or others). On the one hand, proteomic maps can be obtained for the whole tissue or for proteins after prefractionation; on the other hand, the modification of proteome can be compared in two or more conditions. In both cases, the main difficulty remains to be the accessibility of proteins of low abundance. However, 2-DE provides a preliminary view of the main proteins involved in the major biological pathways.

Liver plays a key role in the organism. It is a very complex organ. Indeed, it is involved in the metabolism of lipids and sugars, the synthesis of fatty acids and plasma proteins as well as the detoxification of dangerous substances such as ammonia, bilirubin, xenobiotics and drugs [1]. The liver also synthesizes urea to excrete nitrogenous waste. It stores glycogen, which is involved in the regulation of glycemia and other products such as vitamins, and protective and antitoxic substances. Despite the fact that the liver is a central organ in animals, its study by means of proteomic approaches is poorly documented. The first article on the liver of animal of agronomic interest was published in 2003 [2]. Since then, few articles related to this topic were published. This review aims at synthesizing the studies aiming at exploring the liver proteome of several species in order to either map proteins or compare them in various conditions. In this review, the pathological aspects of liver proteome will not be explored.

## 2. Proteome map

The characterization of the whole proteome of a multicellular organism is a challenging task. The difficulty comes from the wide dynamic range of protein expressions that are up to five or six orders of magnitude. The liver is one of the organs (with brain and plasma) selected for the initial phase of the Human Proteome project (HUPO). Several proteome maps were published with a maximum of 2495 identified proteins [3]. Concerning agronomic species, the first liver proteome maps were published, in bovine species, by Talamo et al. [2] and D'Ambrosio et al. [4] by using 2-DE and, in porcine species, by Golovan et al. [5] and Tsujita et al. [6] by using off gel methods.

The bovine (*Bos taurus*) project aims at defining proteomic maps in different tissues (kidney, liver, muscle and red blood cells) and biological fluids (plasma) and at comparing them to set up a bovine proteome database [2,4]. The use of 2-DE allowed the separation of the main soluble proteins for each organ. For the liver, Talamo et al. [2] could distinctly point out four hundred and eighty four spots on gels (reproducibility 84%). Among these spots, one hundred and twelve corresponding to fifty eight proteins were identified by mass spectrometry. The low identification rate (around 23%) might have been due to silver staining or to the low annotation of bovine genome at the time of the study (2003). The identified proteins represented a large number of protein species which can be linked with specialized biochemical and physiological functions. They corresponded to enzymes involved in energy generation, and carbohydrate, lipid, amino acid and xenobiotic metabolism, as well as proteins involved in polypeptide synthesis, folding and cell structure. Later, D'Ambrosio et al. [4] identified one hundred and thirty four spots corresponding to seventy one proteins. They represented the same functions as those presented above. These studies were the first to describe the liver proteome of mammals (other than humans) even though they faced technical limitations due to the use of 2-DE. Another drawback of these proteomic maps is the limited inter laboratory use. Indeed, the same prefractionation procedure of the sample and migration conditions must be followed to allow any comparison.

The major interest of porcine species, except as meat producer, is its similarity at the level of anatomy and physiology with the human liver. So, pig (*Sus scrofa*) is becoming a more and more popular model for biomedical research [7]. The idea of the construction of a catalog of pig liver proteins is to point out similarities and differences in porcine and human physiology. Two studies were published in 2008 [5,6]. On the one hand, Tsujita et al. [6] aimed at building a catalog of salivary and liver proteins to determine if salivary gland progenitors (stem cells) could be possibly used to regenerate the liver. On the other hand, the aim of Golovan et al. [5] was to establish a highly confident list of liver proteins and to compare it to the liver proteomes of mouse and human. The methods used in these two studies differed in the way that the first one used an on-line two dimensional nanoflow liquid chromatography/tandem mass spectrometry (2D LC–LC–MS–MS) based on a dual trap [8], whereas, the second one used isobaric tags for relative and absolute quantification (iTraq) [9]. Tsujita et al. [6] identified one hundred and fifty four liver proteins mainly located in the cytoplasm and having housekeeping functions. The results were also consistent with previous studies where catalase, cytochrome P450 and ornithine carbamoyltransferase were reported to be highly expressed [10–12]. Only forty five proteins were redundant between the salivary gland and liver. Golovan et al. [5] identified eight hundred and eighty proteins located in the cytoplasm (40%), mitochondrion (18%), endoplasmic reticulum (9%), nucleus (9%) and extracellular regions (7.5%). The comparison with Gene Ontology Cellular Component distribution showed an underrepresentation of the membrane and nucleus proteins in the porcine compared to the human proteome. On the contrary, an enrichment in cytoplasmic proteins was observed in the porcine proteome [5]. The porcine liver proteome has also been submitted to the Proteomics Identifications Database (PRIDE, http://www.ebi.ac.uk/pride/) [13]. By using homology derived secondary structure of proteins, human (2495) [3], mouse (2197) [14] and porcine (880) proteins were compared in order to determine the common and species-specific proteins. The results showed that about 80% of the proteins were at least common in two species (Fig. 1) and were mainly corresponding to housekeeping proteins essential for liver function.

## 3. Liver evolution during development and growth

The evolution of liver proteome during embryogenesis and growing stages has been studied recently in chicken [15,16]. Little is known about the global protein expression during embryogenesis. The identification of differentially expressed