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Proteomics in Animal Health and Production

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Abstract: *Proteomics is a powerful tool for the identification of proteins and study their localisation, functions, modifications and possible interactions or complexes they can form. This review presents an overview of proteome studies of various tissues and biological fluids in different farm animals. Proteomics is the key tool to understand the molecular mechanisms behind meat quality in different genetic back grounds or processing technologies. Several studies carried out on the liver proteome of many species either to mapping the proteins or for comparing them in various conditions. The difference in the proteome of tears in various animal species could explain their adaptation to their climatic conditions. Saliva with its hundreds of proteins, peptides, hormones, electrolytes and other chemical compounds might be a good source of biomarkers which helps in assessing stress factors such as malnutrition as well as diseases or ongoing viral infections in farm animals. Saliva proteome of ruminants could be differentiated from non- ruminants. Proteins are key molecules of the milk functional component repertoire and their investigation reveals differential expression in various animals. The study of proteome can provide a deeper understanding of the biochemical, physiological aspects of farm animal biology and it's relation to productive aspects and disease conditions.*

Keywords: *Meat, Milk, Liver, Proteomics, Serum*

I. Introduction

Proteomics is the study of complete protein profile of a cell, a tissue or body fluid at a given physiological or pathological state. Proteome of a cell can be considered as the bridge between genome and cell function. Proteins are essential components of intricate biochemical and metabolic array of pathways that define any organism and its biology. Proteins play an important role in a diverse range of metabolic aspects in all living creatures, from structural roles to energy metabolism regulation, from parasite tolerance to disease response. Proteins are the major components of every productive aspect of farm animals, from feed quality to product certification, from production of vaccines to the studies of immune response, dairy product processing.

Proteomics aims to the analysis of a number of proteins at a time unlike traditional identification of one protein at a time. Unlike "the genome" there is no single, static proteome in any organism instead there are dynamic collections of proteins in different cells and tissues that display variations in response to various conditions such as stress or infectious processes. Hence the characterization of the whole proteome of a multi cellular organism is a challenging task due to the wide dynamic range of protein expression. The most striking feature of farm animal proteomics is the diversity in farm animals, tissues, organs and body fluids as well as products and their processing conditions. This complexity is further increased if farm animal proteomics includes an increasing number of diseases and disease causing agents such as bacteria, viruses and parasites as well as possible vectors of such diseases namely ticks, flies and mosquitoes (1). The end objective of raising livestock is the production of food / protein to meet the human hunger needs. The nutritive value and edibility of animal products is determined by the type of proteins present in it. An important application of proteomics in relation to the food produced from livestock is in the alterations of the proteins following harvest like the alterations in the muscle proteins as it becomes meat and the alteration of milk proteins during the process of cheese formation.

There are two approaches to proteome characterisation namely comparative proteomics and mapping proteomics. Mapping proteomics aims to characterise and make comprehensive databases or cellular proteomes. This is a huge task, partly due to the complex variety of modification forms of most proteins possess and constant changes in proteome with time (2). Comparative proteomics aims to characterise the biological mechanisms that form the link between observable phenotypes and genotypes, thereby making moment by moment snap shots of cellular responses at the protein level (3).

Proteome analysis relies on four major components such as protein separation, identification, characterisation and quantification essentially resorting to mass spectrometry. The proteome experimental approaches have expanded from the classical identification of all proteins to include functional proteomics (quantification and identification of differentially expressed proteins among distinct conditions), identification and characterisation of protein–protein interactions and characterisation of protein post translational modifications (4). Analysis of cellular proteome is a great challenge due to the complexity of proteins in tissues

and cell lysates. There are two broad categories of proteomic approaches in todays proteomic research, gel based assays and shotgun proteomics.

1.1. Gel based proteomics:

A commonly used approach to analyse proteome is two dimensional gel electrophoresis (2D-GE) which combines two dimensions of physical protein seperation, protein isoelectric point in first dimension and their molecular size in second. In the first dimension, the proteins are separated by their isoelectric point on an immobilized pH gradient according to their aminoacid content (5, 6). In the second dimension, immobilized pH gradient separated proteins are separated by their molecular size similar to a conventional SDS PAGE (5) which results in two dimensional arrangements of proteins with a resolution of single protein type. The separated proteins can be visualized on the gel using different stains, for instance commasie brilliant blue (7), silver (8) or fluorescent staining (9). Each of the detected spots on gel theoretically corresponds to one protein species and each cell or condition of cell is associated with specific pattern of spots on the gel. Changes in spot pattern should therefore reflect changes of cellular proteome, for example its metabolism and gene activity under diseased or healthy condition. Quantification of spot intensity is done using automated digital image analysis (10). The protein spots with significant difference are picked and eluted from the gel and fragmented for mass spectrometry by trypsin digestion (11). The molecular weight of trypsin digested protein fragments is then determined using Matrix Assisted Laser Desorption Ionisation (MALDI) (12). This approach uses trypsin fragment pattern, the so called peptide fingerprint (PF) to identify the regulated protein in open source data bases (13). Due to the lack of complete data bases in animals, PFs obtained from proteome studies in animals in most cases compared with peptide masses of human protein data bases.

1.2. Shotgun proteomics:

This approach is also called as Multidimensional Protein Identification Technology (MudPIT) combines two chromatography separation steps with Tandem Mass Spectrometry (14, 15). Chromatography based proteomics seems to be of greater development potential in terms of mechanization and standardisation. Shotgun proteomic approach involves the separation of complex protein extract in liquid phase, thus avoiding some of common problems associated with gel separation of hydrophobic proteins or proteins with extreme mass/isoelectric P^H (PI) and usually provides markedly better coverage. The combination of two chromatographic methods allows for the separation of digested peptide fragments by at least two features, for example charge and hydrophobicity, to reduce the overall protein complexity (16).

II. Studies In Different Tissues:

2.1. .Meat Proteomics*:*

Meat quality traits such as colour, tenderness are found to be associated with proteome changes. The main factor responsible for meat tenderness is the extent of proteolysis of key target proteins with in muscle fibres (17). Protein degradation and protein oxidation are the processes that modify proteins as well as the tenderness of meat (18). The final tenderness of meat depends on the degree of alteration of the muscle structural and associated proteins (19). Meat proteomics has an important application in the forensic detection of fraud in both raw and cooked meats by identifying the species of origin (20). SDS-PAGE based proteomic study identified chicken specific peptides which could be used as biomarkers for the added chicken to pork or beef (21).

Proteome studies have been performed over the last several years on the change in protein expression during the ageing process and in response to different processing conditions. Several proteome studies have been described the difference in proteins that are present in different meat samples. This approach is called as protein mapping. A protein mapping study of bovine Semitendinosus muscle using a combination of 2–DE and mass spectrometry allowed detection of roughly 300 reproducible protein spots (22). A mapping strategy has also been used to compose the proteome expression of sarcoplasmic proteins between 4 different ovine muscles (23).Vastus medialis muscle had the highest proportion of slow twitch fibres which contain highest amount of enzymes involved in oxidative metabolism and stress. Changes in expression of bovine Semimembranosus proteins induced by muscle hypertrophy were studied by proteome analysis (24). The muscle hypertrophy due to the deletion in the myostatin gene causes changes in proteins of contractile apparatus and metabolic enzymes. Based on these changes in proteins expression, proteomics reflects the shifts in muscles from slow twitch to fast twitch fibres. The effect of quantitative trait loci for muscle hypertrophy on sarcoplasmic proteins expressed in four ovine muscles was investigated by using 2– DE and MALDI TOF – MS (23).

Understanding the variations and different components of the proteome with regard to a certain meat quality or process parameter will give knowledge that can be used in optimising the conversion of muscle to meat. In muscle protein, changes during the process of conversion of muscle to meat results in a lot of variability in the eating quality of meat. Biochemical changes which occur upon slaughter of the animal have

dramatic influence on meat quality and on further meat processing steps. These changes are collectively referred to as post-mortem changes. The interaction of pH, temperature and post mortem time affect the biochemical dynamics of early post mortem meat and hence the rate of proteolysis(25). Geesink and Koohmaraie have described how post mortem degradation of myofibrillar proteins is involved in meat tenderness and in particular the degradation pattern of contractile proteins (26).

Jia *et al.* studied changes in muscle proteome in bovine Longissimus Dorsi (LD) and Semitendinosus (ST) muscles immediately after slaughter and after 24 hours storage. In this study 5 proteins were changed in both the muscles namely cofilin, lactolylglutathione lyase, and substrate protein of mitochondrial ATP-dependent proteinase, SP-22, HSP-27KDa and HSP-20KDa. However 15 proteins were changed in either LD or ST muscles. These differences reflect distinct metabolic and physiological functions of different muscles *(27)*. A study on protein expression during different periods of post mortem storage using pork LD muscles revealed that 15 protein spots were changed such as actin, myosin heavy chain and troponin 1 (28).

Changes in proteome profile of meat with changes in preslaughter condtions were also reported. The effect of postmortem storage time and different preslaughter treatments of pigs on LD muscle proteome were reported (29). Krinsten *et al*. reported the proteome changes induced by different preslaughter conditions in a study of compensatory growth in pigs (30).

Six proteins were affected in a proteome study related to tenderness on pork LD muscle (31). Three actin fragments as well as a myosin heavy chain fragement were correlated to shear force. Myosin light chain II and glycolytic enzyme, triose phosphate isomerase were correlated to tenderness (32).

Tender beef in chianina breed was characterised by higher levels of glycolytic enzymes which results in accumulation of glycolytic end products (33). Sayd *et al.* compared the sarcoplasmic proteome in pig SM muscle from two groups of animals having a light or dark meat colour. The proteome revealed that 22 proteins were differentially expressed while the dark muscle had an increased abundance of mitochondrial proteins indicating a more oxidative metabolism the light muscles had an increased abundance of cytosolic proteins which are involved in glycolysis *(*34).

2.2. Liver proteomics:

Liver is a central metabolic organ which is involved in the metabolism of carbohydrates, lipids, proteins and in urea synthesis to excrete nitrogenous waste. It is involved in detoxification of bilirubin, xenobiotics and drugs (35).

The first liver proteome maps of agronomic species were published in bovine species using 2 DE by Talamo *et al. (36)* and D Ambrosia *et al*. (37). Talamo *et al.* reported 58 proteins while Ambrosia *et al.* reported 71 proteins by MS which are involved in specialised biochemical and physiological functions such as energy generation and metabolism of carbohydrates, lipids, amino acids and xenobiotics as well as in polypeptide synthesis, folding and cell structure.

Porcine model has become more popular model for biomedical research because of its similarity in anatomy and physiology with the human liver (38). Efforts has been made to construct a catalog of pig liver proteins with the aim of studying the similarities and differences in porcine and human physiology (39, 40) and Golovan *et al*. observed that about 80% of porcine liver proteins were common to that of mouse and humans.

The evolution of liver proteome during embryogenesis and growing stages has been studied in chicken. Differential expression of proteins may be due to cellular processes involved in activation or repression of development. Differential expression of regulatory proteins, metabolic proteins, proteins involved in embryogenesis and during different stages (9d, 14d, 19d, hatching) of incubation in chick embryo had been reported using 2-DE (41). Wnt and Cerebrus precursor (a protein interacting with Wnt) were found to be elevated during early embryonic development (42) and involved in changing cell proliferation (43*)*. The proteins associated with the embryogenesis showed an increasing expression during development. The high expression of hexokinase-1 and phosphatidyl choline sterol acyl transferase precursor indicates that the metabolism of liver and of the whole organism is enhanced. On the contrary fatty acid synthase expression is low during embryogenesis.

In chicken the liver is the main lipogenic tissue whereas in majority of mammals the adipose tissue also contributes to lipogenesis. In laying hens, the mobilisation of lipids from the liver is high for yolk lipid synthesis (44). A proteomic study conducted on the livers of laying hens at 0, 10, 21, 32 weeks of age revealed thirteen differentially expressed proteins among which twelve were identified. The proteins triose phosphate isomerase, enolase which are involved in glycolytic pathway were found to be down regulated with age. On contrary FAS and malic enzyme which are involved in fatty acid synthesis were up regulated. These results suggest the switching of metabolism from glycolysis to lipogenesis with increase in age (45).

Zheng A *et al*. studied the liver proteome of lean and fat strains of pekin ducks to know the differences in molecular mechanisms involved in liver metabolism between two duck strains. They observed difference in 76 proteins in the liver of two duck lines. Fat ducks strongly expressed proteins related to pathways of

glycolysis, ATP synthesis, and protein catabolism, suggesting enhanced fat deposition rather than protein retention. Whereas, highly expressed proteins in lean ducks improved protein anabolism and reduced protein catabolism, resulting in an enhancement of lean meat deposition. These observed variations between the two strains at the molecular level are matched with physiological changes in growth performance and meat production. This information may have beneficial impact in areas such as genetic modification through the manipulation of target proteins or genes in specific pathways to improve the efficiency of meat production (46).

Ketosis the major metabolic disorder in cattle is characterised by high plasma concentration of ketone bodies and lower blood glucose levels (47).To face the negative energy balance, muscle proteins and body fat are mobilised leading to the accumulation of triacylglycerides in the liver and hepatic steatosis. Proteomic studies of liver in ketotic and normal cow livers using 2-DE revealed differential expression of seven spots by (48), fifty seven spots by (49). A global proteome of liver was analysed by Kuhla *et al*. to investigate the changes in protein expression in liver, to elucidate the mechanism involved in metabolic adaptation of ruminants to decrease feed intake and mobilisation of body fat reserves. Fifty nine spots were found to be differentially expressed of which thirty five could be identified by MS which are related to lipid metabolism (50). The enzymes Acyl co-A dehydrogenase, Acyl co-A acyl transferase, and β hydroxyacyl co-A dehydrogenase type-2 were all down regulated in ketotic cows and in feed deprived cows.

Liver and mammary gland metabolisms during lactation in cattle were confirmed by the proteomic studies using 2-DE and mass spectrometry (51). Milk proteins α S1, α S2, β and κ-casein, α –Lactalbumin, lactoterrin, β – Lactoglobulin A & B and proteins required for lactose synthesis were detected only in lactating mammary gland. In contrast four enzymes required for urea synthesis and glycogen phosphorylase and gluconeogenic enzymes propionyl Co-A carboxylase, PEP carboxykinase were detected in liver. Pyruvate carboxylase was approximately four times more in liver than in mammary gland. This is in agreement with the metabolic roles of liver for conversion of ammonia to urea, release of glucose into blood, in confirmation of pathways for hepatic synthesis of glucose from propionate.

2.3. Studies in biological fluids

2.3.1. Tears

Proteins in the tear film play an important role in the maintenance of ocular surface, such as protecting the external surface from pathogens and modulating wound healing process (52, 53, and 54). Campos CD *et al*. reported the reference map of proteome of pooled dog tears using 2-DE & MS (55). They reported the presence of major canine allergen protein which is analogous to lipocalin in human tears and also little detectable lactoferrin or lysozyme in dog tears was compared with human tears. A proteome analysis of rabbit tears during corneal wound healing revealed that the level of rabbit defensins (NP-1 & NP-2) elevated after wounding and returned to normal levels by the time of corneal abrasion healed (54).

Seasonal variation in the composition of proteins of camel tears collected during the summer and winter seasons were reported including lactoferins and VMO1 homolog (56). The presence of certain proteins like VMO1 homolog in camel tear film perhaps help the camels to keep ocular surface healthy and to withstand the ocular discomfort caused in desert environment (57).

2.3.2. Saliva

Saliva is a watery fluid containing a complex mixture of proteins, ions and other organic compounds produced mostly by the salivary glands with small portion originating from blood. The connection between local and systemic sources makes saliva as an important fluid to search for biomarkers of diseases or in particular to study a physiological status (58). The use of animal saliva in disease diagnostics requires the characterisation of salivary proteome under healthy conditions. Studies of characterising livestock salivary proteome have started recently and several proteins identified in sheep, goats (59), cattle (60, 61) and pigs (62). Proteins such as amylase or cystatins are absent in ruminant parotid saliva proteomes which may be due to their starch free dietary regimens whereas they are highly abundant in omnivores such as humans as well as in rodents (63).

The secretary processes in salivary glands are influenced by nutrition. Morphological and biochemical studies showed that a protein deficiency leads to an impaired salivary protein secretion (64) and vitamin-D deficiency affects salivary secretion in rats (65). It was reported that iron deficiency in rats lowers the salivary peroxidase activity (66). Ascorbate deficiency significantly reduced the expression of salivary proteins such as amylase and proline rich proteins in guinea pigs (67).

2.3.3. Serum

A study on protein modifications in horse plasma after prolonged physical exercise revealed that prolonged exercise affects plasma proteins involved in inflammation, coagulation, immune modulation, oxidant / antioxidant activity and vascular damage. Most of these changes occurred immediately after the race and returned to control values with in 24/48 hrs and it is considered as a prompt adaptation to stressful end (68).

Proteomic analysis of cow serum from three different production systems revealed oxidative stress response as the main adaptive physiological mechanism. In an attempt to identify new stress / welfare markers using DIGE labelling, 2-DE and MALDI-MS, 15 proteins were identified which are involved in the oxidative stress pathway (glutathione peroxidase and paraoxonase (PON-1), the acute phase protein family (α-HSG), and the complement system. Results indicated that a combination of antioxidant enzymes such as GPx together with HSG, cholesterol and faecal cortisone could be used as an appropriate biomarker profile to assess welfare in challenging conditions in cattle (69). Among the inflammatory proteins up regulated in clinical mastitis, vitronectin is over expressed in both sub clinical and clinical mastitis indicating a strong bacterial infection. This suggests vitronectin as an important mediator in the pathogenesis of the onset of mastitis as well as a valuable marker for diagnosis of the subclinical form of the disease (70).

2.3.4. Milk

Proteins are key molecules of the milk functional component repertoire and their investigation represents a major challenge**.** Proteins in milk such as caseins contribute to the formation of miscelles that are different from species to species in dimension and casein type composition. They are an integral part of the MFGM (Milk Fat Globule Membrane) that has been studied exhaustively studied in recent years. Different types of caseins with different concentrations in cows, sheep, goat and buffalo were reported by Bramanti *et al*. (71). Caseins in equine milk were analysed by Miranda *et al*. (72) concluding that it is most similar to human milk, and could be considered a good substitute of cow's milk for many children with cow milk protein allergy.

Ajay kumar *et al*. (73) studied milk protein polymorphism in Indian goats by proteomic approach and reported the complex heterogeneity in goat milk proteins in different breeds. This complexity is the consequence of post translational modification and due to the presence of numerous genetic variants of proteins and bioactive peptides in milk. Smolenski *et al*. (74) identified 15 proteins involved in host defence in a proteomic study of bovine milk using 2-DE/MS.

One of the most investigated animal pathologies through milk proteomics is mastitis. Eventhough different proteomics approaches were applied to study mastitis in milk, a ready to use proteome biomarker in milk is yet to obtain. In a proteomic study of normal and mastitic whey, Hogarth *et al*. (75) reported an increased concentration of proteins of blood serum origin as serotransferrin and albumin, while concentrations of the major whey proteins α-lactalbumin and β-lactoglobulin were reduced in mastitic whey.

III. Conclusion:

Proteomics can be used specifically to analyze biochemical host-parasite interaction and immune responses of the host to parasite. Proteomic investigations are key to the understanding of biological processes during animal growth, development production and diseases conditions. Proteome analysis has become an important tool for explorative analysis of molecular mechanisms in physiology and pathology (76). Proteomic analysis of metabolic pathways indicates that changes in enzyme concentration often accompany alterations in metabolic output.

Although the genetic background is important, the major contribution to meat quality is caused by processing and environmental conditions. Thus proteomic could be the tool to reflect the important mechanisms and contributions to development of a satisfactory meat quality (77). Proteomics has the potential to shift the understanding of molecular mechanisms underlying meat quality to a great leap forward. Scope of proteomics in animal science is broad and includes the characterisation and monitoring of changes that take place during the growth, development and other physiological processes. Proteomics also serves as key to increase knowledge on the biology of infectious diseases. Disease related application of proteomics includes the ability to identify biomarkers of disease for identification of subclinical conditions, while identification of vaccine candidates is an area of major potential value. Understanding of the molecular mechanisms implied in most of the infectious and parasitic diseases through proteomics contribute to finding of new drugs for treating them.

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