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Proteomics in veterinary science: Opportunities and challenges

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Abstract
The term proteomics refers to the large scale study of proteins, including their structures and functions. A proteome is much more complex than the encoding genome, and the proteins are present across a broad dynamic range as the expression of proteins are regulated in response to various external stimuli. Proteomic analysis involves separation, identification and characterization of complex mixture of proteins. The classifications of proteomics are bottom-up approach and top-down approach as well as label based and label free proteomics. Identification and characterisation of proteins or peptides are done with the help of mass spectrometer and bioinformatics tools. Animal/veterinary proteomics is an evolving field which holds a great promise not only for fundamental and applied discoveries regarding biology and pathology of domestic species, but can also be implemented in comparative applications of human diseases research. While there are certain technical limitations in the expansion of the field, they can currently be circumvented and in the future mastered with a greater participation of proteomic experts, which will in turn drive the accessibility of species-specific reagents, data volume expansion in bioinformatic databases, and increased funding.

Keywords: Proteomics in veterinary science, pathogen proteomics, avian proteomics

1. Introduction
Proteome can be defined as the set of proteins expressed by the genetic material of an organism under defined environmental conditions. The term proteomics refers to the large scale study of proteins, including their structures and functions. This field has emerged in less than 2 decades and has developed rapidly, driven by improvements in technology and by the need for analytic approaches that can deliver global protein characterization.
A proteome is much more complex than the encoding genome, and the proteins are present across a broad dynamic range as the expression of proteins are regulated in respond to various external stimuli. Not just that it is dynamic but the unique chemical properties and handling requirements of proteins make it more difficult to work with than DNA. In addition, small quantities of a particular DNA segment can be expanded greatly using readily available enzymes, whereas protein quantities cannot be increased. This is particularly troublesome when one considers that many of the proteins regulating important cellular processes are present in only a handful of copies per cell. Despite of its remarkable significance in the field of veterinary and animal sciences, proteomics has been limited in these disciplines due to a number reasons, including cost, lack of good genomic data of the targeted species and lack of awareness about the immense potential of this technology among veterinary scientists.

2. Proteomics
Proteomics involves the resolution of a complex mixture of proteins into components that can then be characterized. Characterization primarily involves identification but can also involve relative quantification or further characterization to reveal post translational protein modification. Protein characterization is performed by mass spectrometers, which are generally fed proteins after some initial fractionation or separation.

3. Proteomics in veterinary science: Opportunities
Proteomics can be used in all areas of animal health, production and welfare assessment. It has been used to characterize host-pathogen interaction, in reproductive health assessment, to study dynamics of muscle growth, learn more about alteration in fish muscle and in meat maturation and so on.

3.1 Proteomics in ruminants

Proteomics encompasses new and emerging technologies that will facilitate sustainable animal production, quality and welfare. Given the economic importance of dairy farming, the majority of proteomic studies in bovines have been carried out to elucidate pathogenic mechanisms of mastitis and endometritis. In addition, proteomics has been applied to identify markers of stress in order to monitor animal welfare as well.

Most proteomic investigations of bovine mastitis, using strategies including 2DE followed by matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF)/MS and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), have been performed on bovine milk because of the relative ease of sample collection (Boehmer, 2011) [1].

The techniques have been used to evaluate the modification of milk proteins during mastitis in cows with naturally occurring infection as well as in experimentally induced coliform mastitis (Danielsen *et al*., 2010) [5]. A study found significant increases in serum albumin and transferrin, concurrently with marked decreases in caseins, β-lactoglobulin and α-lactoglobulin, in the whey from cows with mastitis, suggesting that the transport of serum proteins into milk was because of the failure of the blood–milk barrier.

Smolenski *et al*., (2007) [27] identified apolipoprotein A-I (apo A-I), cathelicidin-I, heat shock 70kD protein and the acute-phase protein serum amyloid A (SAA) in milk fractions from cows with naturally occurring mastitis, indicating a local host response to infection in the mammary gland. Another acute-phase protein (APP), α-1-acid-glycoprotein, was identified in normal and mastitis whey samples during a proteomic analysis in cows experimentally inoculated with E. coli. In another recent study, the serum proteome profile in cows with naturally occurring subclinical and clinical mastitis was investigated with three different yet complementary approaches in order to identify differentially expressed protein markers that are useful for early recognition of subclinical mastitis (Turk *et al*., 2012) [30]. These data indicate the involvement of the acute-phase response, oxidative stress, complement activation, protease inhibition and lipid metabolism by the innate immune system to combat infection by pathogens.

Initial investigation into the milk fat globule membrane protein has been followed by analysis of milk exosome proteins (Reinhardt *et al*., 2012) [23] and a combined investigation of the milk fat globule membrane, exosome and whey proteins in S. aureus mastitis (Reinhardt *et al*., 2013) [24]. A total of 300 milk proteins were identified with links to host defence, with 94 being differentially regulated in mastitis.

3.2 Pathogen proteomics

Proteomic technologies have also been used to provide novel approaches and insights into the pathogenic mechanisms of bacterial infection in farm animal diseases, which offer unique opportunities to study the proteome of bacterial pathogens during infection. A limited number of proteomic studies have focussed on pathogen responses during clinical intramammary infection. Taverna *et al*., (2007) [29] discovered major membrane-associated proteins of *Staphylococcus aureus* isolates that could be involved in the recognition of mammary epithelial cell receptors.

2D electrophoresis was also applied to investigate the virulent state of *Mycobacterium avium paratuberculosis* in which a direct comparison of the proteomes of *M. avium paratuberculosis*, scraped from the terminal ileum of ovine paratuberculosis cases, was made to the identical strain grown *in vitro.* This study identified a set of 10 proteins whose expressions are upregulated during natural infection (Hughes *et al*., 2007) [11], which may have implications for biomarker studies and therapy design strategies. Furthermore, proteomic technologies are compatible with novel extraction procedures to enrich for bacterial hydrophobic outer membrane proteins expressed during infection. Finally, the continued development of novel proteomic approaches such as Capillary Electrophoresis-Mass Spectrometry (CE-MS) have the capability to identify panels of peptides that can be used for disease diagnosis and for differential diagnosis of the causative bacteria of the infections of the mammary gland.

3.3 Avian proteomics

An interesting field of application of proteomics is also the study of the pathogenesis of infectious disease affecting the avian species. The importance of this topic ranges from the economical aspect to identifying new biomarkers of vaccines. This also includes the need to study some avian diseases as a zoonosis, for example, avian flu, where human host adaptation signatures have been identified and responses to the virus characterised in mice and chicken (Sun *et al*., 2014). Proteomics has been already utilized to study the pathogenesis of herpes viruses (Kunec, 2013) [13], with a special focus on Marek's disease (Hu *et al*., 2012) [10], which is of particular interest as a model for human tumours (Buza and Burgess, 2007) [2].

3.4 Proteomics in aquaculture

Aquaculture has been ongoing for centuries, but this industry has undergone rapid and extensive expansion because of the rapid growth of the average seafood consumption per person in the last 50 years. To accommodate this demand, aquaculture companies are now breeding fish to improve traits such as their growth rate, conversion of feed into muscle, disease resistance, fertility and other features associated with food quality. Nevertheless, one of the main challenges faced by this industry is its impact on environmental sustainability where clearly a public intolerance to any potential new source of pollution or the further degradation of the natural environment may act as a drawback.

Proteomics application in aquaculture is mainly focussed on nutrition, welfare and health management, as these have proven to be major constraints to an efficient production in aquaculture systems (Rodrigues *et al*., 2012) [25]. With regard to the nutrition source of farmed fish, there is a recent trend to move away from the traditional use of marine-harvested resources towards a diet-containing vegetable protein and oil sources. Although this reduces the impact on the marine-based food source, the growth rates and feed efficiency are compromised. However, proteomics is contributing greatly to a better understanding of the metabolic pathways affected by these dietary changes, as demonstrated in species like rainbow trout, Atlantic Salmon, Gilthead seabream or Diplodus sargus. These studies were mainly focussed on fish liver and muscle with identified protein responses involved in glycolysis, amino-acid catabolism, energy and lipid metabolism, oxidative stress or the immune system.

Fish diseases are responsible for the main economic losses in aquaculture. These diseases are mainly caused by viral,

parasitic and bacterial infections and significantly affect the production yield worldwide. Several pathogen detection methods (traditional, immunological, molecular, etc) have been extensively used, with vaccination being the main research area for disease prevention. Proteomics techniques have been assisting with this problem, especially at the level of development of new vaccines and disease diagnostics. The proteome analysis of the envelope proteins of the pathogen Iridovirus, which is responsible for the high mortality in cultured Grouper and also present in other Southeast Asian farmed species, were studied (Zhou *et al*., 2011) [33].

Proteomics is also an extremely valuable tool in assessing fish welfare through the development of new aquaculture practices that ensure that farmed marine animals can be reared in an environment that optimizes their capacity to cope with unavoidable challenges/stress, thus enhancing their state of welfare and health. The main target organ to be analysed is the liver, providing a window to their metabolic status, or body fluids like blood plasma that is easily retrievable from the live animal. Stress-related studies mostly focussed on the correlation between environmental sources of stress in aquaculture with proteome changes. They include high stock densities, handling and preslaughter stress (Morzel *et al*., 2006) [19]. Studies focussed on the analysis of plasma proteins have concentrated on the detection and validation of welfare markers, with several proteins like microglobulins, macroglobulins, apolipoproteins, α1-antitrypsin, transferrin, plasminogen and complement system proteins among others being identified as possible candidates (Kumar *et al*., 2009).

3.5 Parasite proteomics

The field of parasitology has been quick to exploit emerging proteomics technologies. The investigations have followed two directions. On one hand, proteomics focussed on the 'parasite', trying to identify the expression pattern of parasites, which is particularly challenging because of their different development stages. A second research direction followed the 'host' aspect, thus focussing on the complex dynamics that underlie the interaction between host immune system and parasite. Most, if not all, parasite antigens utilize the change of surface post-translational modifications (PTM) to modulate and possibly evade the host immune response. PTM are involved in many important molecular recognition processes including invasion, adhesion, differentiation and development (Frenal *et al*., 2014) [7].

The advances in immunoproteomic techniques will provide new insights into the 'host' perspective of parasite infections, in particular for what concerns both innate immunity and biomarker discovery for diagnostic applications.

3.6 The metaproteome

All those recent findings demonstrated the enormous impact of proteomics on the development of more accurate and cost-effective diagnosis and prognosis of animal farm diseases. There has already been movement from the single-parameter biomarker strategy to the proteomic-based multi-parameter diagnostic approaches.

However, metaproteomics, also known as the proteomics of the environment or of one particular community, is still an almost unexplored frontier to conquer in animal science. Metaproteomic analysis could provide identification and relative quantification of protein and protein families recovered directly from environmental samples or from a concrete ecosystem. This methodological approach aims to move from a functional understanding of a single species proteome elucidating the proteomic complexity from a larger community.

Today, the latest MS technology in combination with sequencing capabilities and bioinformatic analysis has opened up new opportunities for translating metadata analysis into assessment of a health status (Erickson *et al*., 2012) [6].

There are several advantages to consider: (i) metaproteomics could offer a phenotypic profile on samples preserving the metaspecies environment; (ii) alteration in the microbial ecosystem (saliva, digestive tract, mammalian glands) in farm animals would not only provide functional information for diagnosis but also aid in evaluating stress responses; (iii) it could be of great interest to evaluate gastrointestinal function in ruminant and gastrointestinal disorders.

The main disadvantage is that the developments in metaproteomics would still be linked to growth, achievements and methodological developments of metagenomics. In conclusion, this powerful new methodology would provide a deeper understanding of the farm animal microbiota in the context of functional host–microbe interactions and elucidate the molecular mechanisms linking the microbiome to host physiology.

3.7 Proteomics in animal products

3.7.1 Egg proteomics

Very limited proteomic research has so far been presented regarding quality control in chicken eggs. In particular, Qiu *et al*., (2012) investigated, through a 2DE-proteomic approach, the modification of egg proteins during storage. They described the differential proteome profile at three different storage temperatures (4°C, 20°C and 37°C) for 15 days. The most important result obtained was the degradation of albumin in relation to higher temperature, with the formation of a lysozyme–ovalbumin complex. Furthermore, the relative quantity of clusterin (apolipoprotein J) decreased with the same trend of increasing storage temperature, and it could, therefore, be used to assess egg quality.

Another study applied LC-MS/MS proteomics to investigate the eggshell cuticle proteome, which represents the real barrier against the external environment and as a defence against mainly bacterial assault. This study of the protein composition of egg cuticle represents a milestone and highlights several parameters that can be used to assess egg quality. This is particularly important as even partial damage to the cuticle exposes eggs to microbial contamination from food-borne pathogens. Among the 47 proteins identified, two major proteins – namely, a Kunitz-like protease inhibitor and ovocalyxyn 32 – are known to present antimicrobial functions. These findings may be relevant for prediction/selection of eggs with increased resistance to food-borne pathogens.

The egg white protein has been examined by proteomics. Wang *et al*., (2012) [31] used a combined 2DE and LC/MS/MS proteomic approach to explore relative differences of egg white proteins across six different egg varieties. They found for the first time a quiescence precursor protein in eggs, previously identified only in chicken mesenchymal and fibroblast cells. These authors concluded that the proteome of different egg varieties has the same components; however, the relative abundance of individual proteins does vary between the different egg varieties.

3.7.2 Milk

Calvano *et al*., (2013) [4] described a rapid and sensitive method to detect adulteration in milk, in particular to detect mixtures of powdered milk in liquid milk, both in raw and processed products. The same results can be obtained with 2DE-based proteomic analysis, but MALDI-TOF-TOF analysis is a reliable and fast method for this purpose. In particular, they identified diagnostic peptides of powdered milk with sensitivity of <1%.

Nissen *et al*., (2013) [20] described a powerful combined prefractionation method to characterize the bovine milk proteome. Authors were able to identify new proteins, and their data were supported by ELISA validation. The combination of accurate prefractionation methods, 2D-based proteomics, LCMS/MS and ELISA can efficiently overcome the problems of measuring minor milk protein components, despite the large dynamic range of milk proteomes.

Caira *et al*., (2013) [3] described different typical peptides useful to detect different types of milk and adulterations through the detection of αS1-CN variants. Furthermore, they set up a flow injection analysis electrospray ionisation quadrupole TOF analysis before dephosphorylation of casein that could also be used to assess different Mediterranean breeds.

Negative energy balance (NEB) in cattle directly influences milk composition, with respect to both proteins and lipids. Lu *et al*., (2013) applied a proteomic and metabolomic approach to study NEB in milk. These authors showed that milk from these cows has an increased quantity of acute-phase proteins, galactose–1–phosphate and unsaturated fatty acid, in comparison with milk from cattle with a good energy balance. These observations have provided some insight into the potential mechanisms involving stomatin and galactose-1-phosphate in NEB dairy cow's milk and demonstrate a relationship between metabolism and the quality of milk.

Although safety of dairy products is a prerequisite for the industry, food-borne diseases continue to be one of the most important causes of disease and fatalities in humans. Proteomics can help improve the detection of pathogens in food samples – for example, MALDI-TOF MS for diagnostic microbiology is a successful example of how proteomics can win this global challenge. One study was focused on detection of one of the more dangerous food-borne pathogens, *Listeria monocytogenes*, directly on selective-enrichment broth. MALDI TOF MS was successfully applied to detect pathogens in blood, but the real challenge was to apply these methods in complex pathogen systems as in food matrices using the SARAMIS database, with an algorithm optimized for the rapid detection of Listeria-contaminated food matrices.

3.7.3 Cheese

In dairy products, especially for cheese, there has been a rapid increase in research using proteomics to study processes such as cheese maturation in order to improve the quality of production. For example, Hinz *et al*., (2012) [9] used a 2DE-based proteomic approach to correlate the differences in the proteolysis of milk proteins during lactation stages. They correlated the proteomic pattern of the relative production of cheddar cheese derived from different time points of the lactation stages. Interestingly, they identified some proteins that could be useful to assess seasonal quality.

Wedholm *et al*., (2008) [32] described protein markers of the cheese yield through proteomic analysis. In particular, they highlighted several proteins related to the production as a specific β-CN fragment, an isoform B of β lactoglobulin, and other whey proteins as lactoferrin and vitamin D-binding proteins. However, the roles of these proteins were not completely explained.

3.7.4 Meat

3.7.4.1 Poultry

Although regarded as a major food source for humans, proteomics in poultry and fish science is still lagging behind that for other livestock species. Proteomics in poultry has focussed on basically two fields of research, meat quality and the study of infectious diseases. Differences in raw and cooked poultry meats were determined by means of proteomics (Montowska and Pospiech, 2013) [18], which also provided the tools to identify new protein markers associated with slow and fast growth rates of a different genetic line.

3.7.4.2 Porcine and bovine:

Proteomics was also useful to unravel the molecular basis of some protected designation of origin meat products paving the way for future application of proteomic techniques in food quality certification. The derangement of post-mortem alteration such as those that drive to pale soft exudative meats can also be analysed by proteomics (Molette *et al*., 2003) [17]. Given the background of the growing sensitivity of consumers and of policy makers on animal welfare, future applications of proteomics for the identification of biomarkers of stress and welfare after transport, for example, or pre-slaughtering procedures, are also envisaged (Hazard *et al*., 2011) [8].

A number of studies using proteomic tools have been undertaken to analyse why different pig breeds are associated with extensive differences in meat quality. One example is the comparison of the Italian Casertana pig breed with the Large White breed using both proteomics and metabolomics. The Casertana breeds grow slower and have more backfat and intramuscular fat than do fast growing and leaner breeds like the Large White. The slower post-mortem pH decline in meat from Casertana pigs and differences in metabolic rate were supported by the differences in protein abundances and levels of specific metabolites. Higher levels of glycolytic enzymes and increased lactate accumulation were observed in the Casertana breed. On the other hand, meat from the Large White had higher expression of genes involved in cell cycle and muscle growth, also supported by proteomics. Furthermore, the metabolomics studies revealed an increase in Glutathione and Glutathione disulphide in Large White compared with Casertana pigs.

Post-mortem changes in the porcine muscle proteome include both metabolic enzymes and myofibrillar proteins. In addition, similar studies performed in beef show that many metabolic enzymes, cellular defence and stress proteins change in abundance post-mortem (Laville *et al*., 2009) [14]. The proteome analyses support a shift in energy metabolism post-mortem. Aerobic metabolism continues or a short period after slaughter in the muscle, demonstrated by increased abundance of enzymes involved in the glycolytic pathway. The resulting production of lactate and protons contributes to the pH decline. Several studies have shown that the abundance of heat shock proteins (HSP) 27 and 70, both known inhibitors of apoptosis, is decreased, eventually increasing apoptosis in postmortem muscle.

For beef, tenderness is the most important eating quality parameter determined by consumers (Miller *et al*., 1995). The search for reliable markers of tenderness in beef using various

proteomic approaches has been a major focus for several research groups over the last decade (Sierra *et al*., 2012). Several markers have been proposed, and these are involved in different biological pathways or functions such as myofibril structure, proteolysis, oxidative stress resistance, apoptosis or energy metabolism. Proteins belonging to the HSP27 and HSP70 families are among the most promising tenderness markers so far, although these proteins are involved in a number of different cellular responses. In addition to their anti-apoptotic role, these proteins are protectors of myofibrillar proteins like desmin, actin, myosin and titin.

3.7.4.3 Proteomics of processed meat

Production of cooked ham involves several processing steps such as injection of brine with different salt content, and tumbling of the muscle, before the final cooking step. Pioselli *et al*., (2011) [21] observed significant differences in myofibrillar muscle protein composition of the exudates when using different salt concentrations and tumbling times. An initial salting step is also a central part of the processing when producing dry-cured hams. Proteins are released in the exudate formed in the initial salting step of Italian Parma ham production, and the processing conditions influence significantly the release of myofibrillar proteins in the exudates. Most of these proteins belong to the myofibrillar protein fraction. Release of amino acids and peptides by proteolysis of myofibrillar proteins is important for taste and odour in dry-cured hams. Several peptides derived from actin, myosin light chain and creatine kinase are among the peptides released during ripening of Spanish dry-cured hams.

3.8 Proteomics in companion animals

Companion animals and humans frequently suffer from similar diseases, such as cancer, heart disease, kidney disease, and obesity, which make them excellent models for comparative proteomics research.

A recent proteomic study of muscle samples from Golden Retriever muscular dystrophy (GRMD) dogs and healthy controls by ICAT profiling revealed that proteins involved in metabolic pathways were decreased in abundance in GRMD muscle, with defective energy metabolism as a hallmark of the disease. This animal model of comparable to Duchenne muscular dystrophy (DMD) can provide new insights into the nature and time course of molecular derangements of dystrophic muscle.

It is recognized that dogs often develop tumors similar to those in humans, as they are exposed to the same mutagenic and carcinogenic threats. For example, immunoproteomic approach led to the discovery of four disease-associated autoantigens in dogs with breast cancer which has been reported to elicit autoantibody response also in the human breast cancer.

Due to the similarities in anatomy and physiology, as well as the high degree of human heart proteome conservation, dogs (along with pigs) are the most commonly used animals in human cardiovascular disease research. It is showed that 6575 from 44 272 proteins identified in human myocardium, based on data available from Uniprot database, shared the minimum 90% of the amino acid sequence both with the dog and the pig.

Heart diseases are one of the most common diseases of pet dogs and cats, affecting 10–15% of all dogs and cats. A recent study on serum of dogs with canine idiopathic dilated cardiomyopathy (iDCM) using label-based quantitative proteomics approach revealed potential heart tissue remodeling markers which could also be of relevance in human iDCM.

Proteomic analyses of different biological fluids in healthy companion animals have been performed; allowing the creation of proteome catalogues which can serve as a reference point in the new studies. Proteomes of canine serum, urine, saliva, seminal plasma, tears, cerebrospinal fluid, bronchoalveolar lavage fluid, and follicular fluid were characterized using proteomic methods such as 2DE or gel-free LC-MS.

The identification of biomarkers related to diseases provides diagnostic tools as well as potential targets for drug development and vaccine candidates. For example, a gel-based proteomic approach with enrichment of low abundance plasma proteins was employed for finding biomarkers associated with canine babesiosis; while gel-free, isobaric-based quantitative proteomic approach enabled the identification of new potential biomarkers for the treatment monitoring of canine leishmaniosis. The translation of these results into technology for use in routine veterinary practice is keenly anticipated.

While canine models of human diseases have been used in certain extent in proteomic studies, feline diseases are underutilized which is a missed opportunity as they can serve as good models of type 2 diabetes mellitus or inherited muscular dystrophies disorders, being clinically similar to those in humans. One of the examples where cats were used in a proteomic study is research on familial hypertrophic cardiomyopathy (HCM). Cats with HCM represent a good intermediary model between the mouse models and humans to study disease progression, development of the congestive heart failure, and different therapeutic strategies.

4. Limitations

The trend in proteomics has moved from a gel-based technology that could be available in almost every laboratory at relatively affordable costs to a mass spectrometry-based technology that implies the use of multimillion euro/dollar instruments that in turn require very specialised staff and have to run 24/7, 365 days a year in order to compensate for the initial investment. Such instruments cannot be available to the majority of institutions that conduct farm animal research. They may however be available through centres with hi-tech proteomic platforms that analyse samples from different countries, regions or even the whole world. This is perhaps one of the most important challenges to researchers in animal sciences. Indeed, a successful connection to a proteomic platform may be difficult to achieve for a researcher that is not familiar with proteomic principles.

Another difficulty relates to the high requirements for funds that are necessary to access such platforms. In fact, farm animal research project budgets seldom include the amounts required to analyse all samples in a trial. As such, a compromise between statistical significance and budgetary limitations may have to be found.

Proteomics in farm animals has also to struggle with some technical limitations. Probably the most significant is related to the multispecies nature of the research. Indeed, if proteomic research in sequenced species like cattle (*Bos taurus*), pig (*Sus scrofa*) or chicken (*Gallus gallus*) tends to lead to very complete results, research with less known species like goats (*Capra hircus*), horses (*Equus caballus*) or turkeys (*Meleagris gallopavo*) and geese (*Anser anser*) tends

to lead to very incomplete results due to the lower representation of the latter in protein (and gene) databases. It is likely that in the future sequencing of such species will become a reality. The lowering of sequencing costs experienced in the last decade does point that way. To contribute to solving this problem and from an individual research initiative perspective, it is important to supply sequencing data obtained and insert it in public databases, so it may be accessible to all researchers in the field.

Other technical limitations have to do with the nature of the proteinaceous material studied. It is relatively straightforward to find protein extraction protocols for certain tissues such as the muscle or liver; for others like wool or honey, adequate protocols are needed. It is therefore important that researchers publish their findings in international peer-reviewed journals, highlighting protocol aspects in detail so that other researchers may adopt such works contributing to strengthen the farm animal proteomic research community. It is equally important that journal editors and reviewers recognise the importance of comparative proteomic data from multiple species so that the knowledge base on the animal species used for livestock and aquaculture production keeps pace in dissemination with the advances in technology.

5. Future technical advances for animal proteomics

The evolution of proteomics this last decade is strongly correlated to both technology and bioinformatics advances. At present, proteomic studies allow the high-throughput analysis of thousands of proteins leading to a huge amount of data. The rise of data-independent techniques on increasingly sensitive MS reinforces this trend. MS data need to be processed by adequate bioinformatics tools in order to extract biologically relevant information. When applied under 'good practice' and following the up-to-date guidelines, detection, identification and quantification of proteins can be achieved with precision and reproducibility. Furthermore, data interpretation relies on knowledge of proteins that is tightly related to genomics, transcriptomics and metabolomics data. It is now relatively straightforward to apply tools developed for human and mouse genomes to a few well-assembled and annotated livestock genomes. However, there are several unsolved issues in making these genome resources relevant to agriculture. One important challenge is the management and representation of thousands of variant genomes per species. Existing efforts to adapt bioinformatics to the needs of different applications of biotechnology have remained dispersed, missing the integration. These application criteria characterize resources for major bio-medically relevant organisms and lack attention to the usability needs that correspond to different stakeholders such as breeders, biotechnology small and medium-sized enterprises, food industry and environmental or marine biology monitors. Protein functional annotation and omics data integration still need rigorous exploration and streamlining.

There is thus a current wave of interest in the application of proteomics to animal research and it is important that the momentum gained is not dissipated but is captured to yield the maximum benefit possible. How can this be achieved? The equipment needed to deliver technology for proteomics is likely to remain expensive for the foreseeable future, with few animal science institutes being willing to devote resources exclusively to proteomics; thus, it is important that centres of proteomic expertise remain open for collaboration with animal research. With the increasing recognition of the importance of research impact to the wider community, such laboratories should become more amenable to collaboration as direct links to public benefit and research impact can be established. Proteomics can identify disease biomarkers and vaccine candidates for economically important diseases of livestock and fish in aquaculture, as well as play a major role in species authentication to reassure consumers of food products on supermarket shelves. Many of these applications have commercial application and it can be expected that veterinary and animal diagnostics, along with vaccine and animal health product companies, will recognize the need to explore the proteome of blood and tissues from experimental or natural disease investigations in order to determine the roles and potential actions of bioactive proteins and peptides.

A growing range of applications for proteomics of domestic animals, evidenced over the experience of the last few years, will extend the demand for expertise to apply the technology as access and the knowledge base increases. The ubiquitous use of 1D electrophoresis based on the Laemmli polyacrylamide gel technique throughout biological research indicates the need for protein analysis. As the use and value of proteomics with greater separation and more accurate protein identification within animal species increases, the potential applications of the method in animal science will multiply. As this occurs, it is likely that both equipment and reagent manufacturers become more familiar with the sector. Production of kits for the major farm animals would be of significant benefit to biomarker discovery in these species.

6. Conclusion

It is clear that there is a need for proteomics to be included in future investigation of animal health, welfare and production. Expertise and motivation have been developed among a cohort of researchers to maximize the potential for future proteomic-based investigations.

Whatever happens there is no doubt that the use of proteomics in animal research has now gone beyond a few, isolated laboratories and can now be seen as a mainstream research tool of benefit across the spectrum of investigations into animal health, production and post-harvest processes. It is to be hoped that national and international funding bodies that allocate research funds will have the foresight to recognize that there is now a window of opportunity with technology.

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