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EDITORIAL

Unveiling the power of proteomics in advancing tropical animal health and production

Mounir Adnane[1] · André M. de Almeida[2] · Aspinas Chapwanya[3]

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Abstract
Proteomics, the large-scale study of proteins in biological systems has emerged as a pivotal tool in the field of animal and veterinary sciences, mainly for investigating local and rustic breeds. Proteomics provides valuable insights into biological processes underlying animal growth, reproduction, health, and disease. In this review, we highlight the key proteomics technologies, methodologies, and their applications in domestic animals, particularly in the tropical context. We also discuss advances in proteomics research, including integration of multi-omics data, single-cell proteomics, and proteogenomics, all of which are promising for improving animal health, adaptation, welfare, and productivity. However, proteomics research in domestic animals faces challenges, such as sample preparation variation, data quality control, privacy and ethical considerations relating to animal welfare. We also provide recommendations for overcoming these challenges, emphasizing the importance of following best practices in sample preparation, data quality control, and ethical compliance. We therefore aim for this review to harness the full potential of proteomics in advancing our understanding of animal biology and ultimately improve animal health and productivity in local breeds of diverse animal species in a tropical context.

Keywords Proteomics · Domestic animals · Multi-omics integration · Sample preparation, biovariability · Challenges

Introduction

Proteomics is an emerging field that aims to comprehensively analyse the complete set of proteins expressed by an organelle, cell, tissue, organism, or population (McArdle and Menikou 2021). Proteomics approaches have been applied to various areas of biology, including human health, plant, and animal sciences (Aslam et al. 2017; Petricoin and Liotta 2003). In domestic animals, proteomics can be employed in many biological fields such as physiology, development, disease, or nutrition, especially in livestock (cattle, pig, horses and small ruminants) as well as poultry where the economic importance is undoubted (Almeida et al. 2021; Almeida et al. 2017; Ferlizza et al. 2015; Ribeiro et al. 2020). A main advantage of proteomics is the provision of a global view of protein expression patterns in an organism. This is critical for identifying new biomarkers, drug targets, and pathways involved in different biological processes (Petricoin and Liotta 2003). The tropics and the subtropics, being regions facing inherent local challenges, are particularly important in the framework of animal production, given the fact that the vast majority of the human population inhabits these regions.

Over the last two decades, mass spectrometry (MS)-based techniques have become prominent and a preferred approach for accurately and comprehensively identifying and quantifying proteins in biological samples (Schubert et al. 2017). These methods have played a pivotal role in advancing our comprehension of cellular signalling networks, shedding light on the intricate dynamics of protein-protein interactions across various cellular states, and enhancing our diagnostic capabilities and molecular insights into the mechanisms underlying diseases. Distinguishing between high-quality and low-quality proteomics

✉ Mounir Adnane
mounir.adnane@univ-tiaret.dz

[1] Department of Biomedicine, Institute of Veterinary Sciences, University of Tiaret, Tiaret 14000, Algeria

[2] LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associate Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, Lisboa 1349-017, Portugal

[3] Department of Clinical Sciences, Ross University School of Veterinary Medicine, Basseterre 00265, Saint Kitts and Nevis

data is generally straightforward. However, precisely defining the boundary between them remains a complex task, mainly due to the frequent use of algorithms and statistical measures that are not always fully comprehended by end-users (Handler et al. 2018).

Proteomics has undergone a remarkable transformation since the early days of Multidimensional Protein Identification Technology (MudPIT) shotgun proteomics in the early 2000s. This approach, once hailed, is now recognized for its notable occurrence of false-positive peptide-to-spectrum matches. A pivotal step forward for the field was the introduction of methods to estimate false discovery rates (FDR), employing reversed or randomized databases within a target/decoy strategy. Recent developments within the field reveal an emerging trend where authors are increasingly expected to include supplementary experimental validation for MS-based data before it gains approval for publication. Furthermore, proteomics delves into the functional relationships between proteins and their interactions with various molecules, such as nucleic acids and small compounds (Iacobucci et al. 2021). Nonetheless, a comprehensive review of contemporary proteomics technologies, their utilization in domestic animals, emerging trends, and associated challenges is warranted.

Proteomics, as well as other Omics, have been used extensively to study biological traits such as growth, milk yield, herd longevity, health, and reproduction of local and genetically selected breeds (Dai et al. 2018; He et al. 2019; Mol et al. 2018; Wang et al. 2022) such as goats (Cugno et al. 2016; Hernandez-Castellano et al. 2016; Parreira et al. 2020; Ribeiro et al. 2023). For diagnostics, proteomics has utility in identifying potential biomarkers of diseases such as mastitis or peripartum insulin resistance in dairy cows (Tong et al. 2020; Yang et al. 2014; Zachut 2015), respiratory diseases in pigs (Chaudhari et al. 2021) paratuberculosis in sheep (Zhong et al. 2011), tropical tick-borne diseases (Cugno et al. 2016) and markers of virulence of tropical tick borne- Cowdriosis disease (Marcelino et al. 2012). Furthermore, proteomics can identify potential targets for novel therapies or vaccines against prevalent and/or emerging animal diseases. For instance, proteomics and bioinformatics analyses described potential drug targets for African swine fever virus (ASFV) (Wohnke et al. 2021). This study compared the proteomes of porcine macrophages and a macrophage cell line infected with ASFV. Here, molecules differentially expressed included proteins involved in immune responses, cell cycle regulation, apoptosis, and autophagy and NF-κB signaling pathway inhibitors (Wohnke et al. 2021).

Proteomics analyses has the potential to revolutionize animal sciences by providing a global view of protein expression patterns and functional relationships. These, in turn, can improve animal health, production, and welfare. Despite these advances, comprehensive reviews focusing on the applications, challenges, and future directions of proteomics in local breeds of tropical livestock are warranted. Moreover, most proteomics studies were conducted in model organisms and not in farm animals. However, in-depth studies in livestock would allow a better understanding of biological, physiological, or pathological processes. This review synthesizes the current knowledge and identifies gaps and opportunities for future research in the field of proteomics pertinent to farm animals, including local breeds in the tropics and the subtropics.

Overview of main proteomics methods

Proteomics technologies and methodologies have revolutionized the way we study and understand complex protein interactions. With the advent of advanced technologies and bioinformatics tools, it has become possible to analyse whole proteomes (Table 1).

Gel-based proteomics

Gel-based proteomics are widely used for protein characterization. Two-dimensional gel electrophoresis (2-DE) is a common gel-based proteomics technique that separates proteins based on their isoelectric point (pI) and molecular weight (Bunai and Yamane 2005) (Table 1; Fig. 1). This technique involves separating proteins based on their isoelectric point and molecular mass using polyacrylamide gels (Ahmad Mir et al. 2023; Magdeldin et al. 2014). The separated proteins can then be visualized using staining methods such as Coomassie blue or silver stain, and subsequently identified using mass spectrometry (MS) analysis.

One of the main advantages of gel-based proteomics is low cost, high resolution, and sensitivity (Chevalier 2010; Scherp et al. 2011). Gel-based proteomics can provide information on protein isoforms and post-translational modifications (PTMs) such as phosphorylation and glycosylation (Zhang et al. 2010). 2-DE technique involves running proteins on a first-dimension gel based on their pI, followed by a second-dimension gel based on their molecular weight. 2-DE has been used to identify numerous proteins in various domestic animal species, including cattle, pigs, sheep, goats, and poultry (Bunai and Yamane 2005). However, gel-based proteomics also has limitations, including difficulty in detecting membrane-bound and hydrophobic proteins, and limitations in dynamic range (Bunai and Yamane 2005; Garbis et al. 2005). In fact, the primary limitation in 2-DE currently is its capability for protein detection rather than achieving optimal protein resolution.

Table 1 Pros and cons of proteomic technologies applicable in veterinary practice. Most techniques in domestic animals allow acquisition of multiple sampling in time series and availability of biological samples in sufficient volume. This facilitates multiple analyses, such that both experimental and natural disease processes can be investigated

Proteomic technique		Pros	Cons	References
Gel-based proteomics	Two-dimensional gel electrophoresis (2-DE) (conventional Gel-based proteomics, Coomassie blue or silver stain	- Separating proteins based on their isoelectric point and molecular mass using polyacrylamide gels - Low cost, high resolution, and sensitivity - Provide information on protein isoforms and post-translational modifications	- Difficulty in detecting membrane-bound and hydrophobic proteins, - Limitations in dynamic range - DNA fragmentation and altered expression of tumour suppressor proteins	(Ahmad Mir et al. 2023; Bunai and Yamane 2005; Chevalier 2010; Garbis et al. 2005; Magdeldin et al. 2014; Scherp et al. 2011).
	Modern fluorescent protein stains (ruthenium bathophenanthroline disulphonate, Coomassie Brilliant Blue)	- Enhancing the sensitivity of detecting proteins facilitating more precise protein quantification	- Relatively high background staining - Nonselective dye particle binding to the gel - Limited linear dynamic range	(Patton 2002; Steinberg et al. 1996).
	Modern fluorescent protein stains (Sypro Ruby, Deep Purple)	Higher sensitivity compared to Coomassie Brilliant Blue	ND	(Harris et al. 2007)
	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)	- Separates proteins based on molecular weight and charge - Cost effective - Doesn't require high technicity	- Time consuming - Limited tissues samples compatible for analysis	(Al-Tubuly 2000; Liu et al. 2014a)
	Gel-Based Liquid Chromatography-Mass Spectrometry (GeLC-MS)	- Addresses challenges posed by limited tissue samples - Efficient protein characterization with minimal starting material	ND	(Makridakis and Vlahou 2018; Ploypetch et al. 2020).
	SDS-PAGE focusing	- Precise separation of small-size fragments - Enhanced resolution of proteins with closely spaced molecular weights - Enhances the accuracy and sensitivity of protein profiling		(Zilberstein et al. 2007a).
	Difference in gel electrophoresis (DIGE)	- Comparison of two or more samples within the same gel - Reducing gel-to-gel variation - Increasing accuracy		(Lottspeich 2009).
Targeted proteomics	Main technique	- Sensitive, precise, and reproducible detection and quantification of specific proteins - Simultaneously quantify 10 to 100 target proteins	- Often allow detection of few dozen selected proteins - Technique is still in continuous improvement - Lower throughput compared to traditional enzyme-linked immunosorbent assay (ELISA) methods	(Aebersold et al. 2016; Borras and Sabido 2017; Yoneyama et al. 2017)
	Selected Reaction Monitoring (SRM)	- Focuses on limited specific peptides chosen for quantifying	ND	(Korbakis et al. 2015)
	Parallel Reaction Monitoring (PRM).	- Simultaneously analyses all fragment ions of a pre-selected peptide - High dynamic range - Exceptional signal-to-noise ratios, - Superior sensitivity and specificity for peptide and protein quantification	ND	(Lawrence et al. 2016; Martinez-Garcia et al. 2016).

Table 1 (continued)

Proteomic technique		Pros	Cons	References
Mass spectrometry-based proteomics	Main technique	- Study protein expression, interactions, modifications, and functions in different conditions	May mask the heterogeneity and complexity of individual cells	(Han et al. 2008; Neagu et al. 2022; Noor et al. 2021)
	Matrix-Assisted Laser Desorption/Ionization (MALDI),	- Identify and characterize proteins and peptides including their post-translational modifications (or protein-protein interactions. - High-speed protein quantification - Visualizing the spatial distribution of proteins and other molecules within thin slices of animal tissue - Reveal molecular changes associated with diseases	ND	(Reyzer et al. 2010).
	Electrospray Ionization (ESI) -MS	- Identify or characterize proteins and peptides, especially those with high polarity, low volatility, or thermal instability - Can be combined with other proteomic techniques to achieve higher resolution, sensitivity, and specificity		(Loo 2000) (Doerr 2013; Xu and Sun 2021)
	Label-based approaches (Isobaric Tags for Relative and Absolute Quantitation (iTRAQ))	- Ability to label proteins in two or more samples with different isotopes - Highly reproducible - Sensitive	- Expensive - Time-consuming	(Afedi et al. 2021; Jiang et al. 2020; Wang et al. 2017; Zhu et al. 2019).
	Label-free approaches	- Faster and less expensive than label-based approaches	- Less accurate due to technical variability	(Noor et al. 2019).
	Label-free spectral counting	- Simple - High-throughput capable, - Useful tool for large-scale proteomic studies.	- Accuracy affected by several factors such as non-reproducibility of results, peptide detectability, incomplete protein digestion, and sample complexity	(Zhang et al. 2006).
	Label-free functional proteomics	- Identifying protein-protein interactions, - detect post-translational modifications, - Detect localization of proteins within cells or tissues		(Monti et al. 2005)
Bioinformatics tools		- Processing, analysis, and interpretation of large proteomics datasets - Identification and quantification of proteins from complex mixtures	ND	(Gligorijevic et al. 2018)
Antibody-based approaches for Validating regulated proteins	Conventional Western bloating	- Concurrent profiling of specific proteins with precision, sensitivity, and speed	- Protocol rather lengthy - Relies on the availability of specific antibodies	(Barkovits et al. 2021; Brennan et al. 2010; Ohara et al. 2006).
	Rapid western blot	- Reduces the time and cost of western blot analysis - Compatible with multiplex analysis - Similar or better accuracy and precision than conventional Western blot analysis	- Specific antibodies are required - Potential for cross-reactivity	(Barkovits et al. 2021).
	Enzyme-Linked Immunosorbent Assay (ELISA),	- Detection of a wide range of proteins in various biological fluids	- non-specific binding	(Breard et al. 2020; Fitria et al. 2023)

Table 1 (continued)

Proteomic technique		Pros	Cons	References
Multi-omics	Metagenomics, metatranscriptomics, metaproteomics, metabolomics, and lipidomics	- Provide a more comprehensive understanding of biological processes and pathways - Investigates local breeds or other species to improve genetic potential of farm animals	ND	(Heintz-Buschart and Westerhuis 2022; Kusumawati et al. 2021; Thomas et al. 2016).
Single-cell proteomics and spatial proteomics	Main technique	- Analyzing the proteome of individual cells - Detailed understanding of cellular heterogeneity and function		(Bagger and Probst 2020; Codeluppi et al. 2018)
	single-cell Deep Visual Proteomics (scDVP),	- Combines high-content imaging, laser microdissection, and multiplexed MS - Identify thousands of proteins in a single-cell slice		(Rosenberger et al. 2022)
Proteogenomics and proteotranscriptomics		- Combine proteomics data with genomic/transcriptomic data - Provide valuable insights into the functional roles of genes and proteins		(Fancello and Burger 2022; Menschaert and Fenyo 2017)
Artificial intelligence and machine learning		- Allow analysis and interpretation of complex proteomic datasets - Automatically detect and correct errors in mass spectrometry data - Peptide identifications and spectral counts - identify clusters of proteins with similar expression profiles - Build predictive models based on proteomic data - Transform proteomic data into intuitive visual representations	- Require expertise in informatic - High performance informatic tools and internet are required - Large stockage drives are required	-

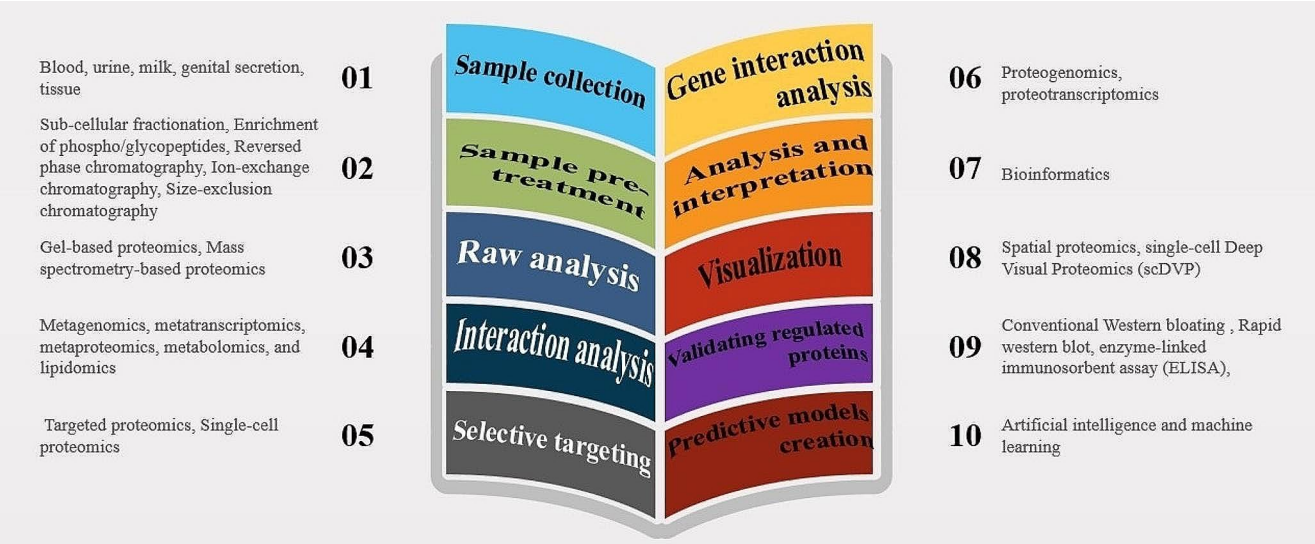

Fig. 1 An illustrated guide to adaptable proteomic techniques flowchart for veterinary applications

The introduction of modern fluorescent protein stains represents a significant advancement, greatly enhancing the sensitivity of detecting proteins within gels and facilitating more precise protein quantification (Harris et al. 2007). Protein stains are pivotal in achieving accurate and quantitative representations of proteomes, with sensitivity and selectivity as critical factors. Historically, traditional methods for detecting total proteins encompassed radiolabelling, Coomassie Brilliant Blue (CBB), and silver staining. Radiolabelling was once the primary post-electrophoretic detection method for proteins in polyacrylamide gels (Patton 2002). However, it has been associated with adverse effects on metabolic cells, including DNA fragmentation and altered expression of tumour suppressor proteins (Hu and Heikka 2000; Hu et al. 2001).

In contrast, the fluorescent family of metal-chelate stains, such as ruthenium bathophenanthroline disulphonate and the well-known Sypro Ruby (SR), have emerged as the

new gold standards for protein detection and quantification (Steinberg et al. 1996). These fluorescent staining methods have proven highly effective. Among the widely used total protein stains, silver staining, which relies on saturating the gel with silver ions that bind to gel proteins and are subsequently reduced to form metallic silver (Oakley et al. 1980). While silver stain offers higher sensitivity than CBB on average, it still faces challenges of limited dynamic range and protein-to-protein variation in staining intensity (Harris et al. 2007; Oakley et al. 1980).

Coomassie is another common nonfluorescent technique. Both CBB and its variant colloidal CB, stands out for being cost-effective, user-friendly, and compatible with mass spectrometry (Patton 2002). However, CBB exhibits some limitations, including relatively high background staining due to nonselective dye particle binding to the gel matrix and a limited linear dynamic range (Patton 2002). Recent reports have highlighted enhanced sensitivity for CBB-based staining when using near-infrared (IR) fluorescence imaging for detection (Luo et al. 2006).

To address these limitations, fluorescent protein detection methods have been developed. One of the most commonly used fluorescent stains is SR, known for its metal-chelate organic complex that interacts noncovalently with primary amines of amino acids, such as lysine. SR has demonstrated significantly higher sensitivity compared to CBB. Another fluorescent stain, Deep Purple (DP), utilizes epicocconone, a natural fungal product, to react with primary amines like lysine residues, producing a fluorescent enamine (Harris et al. 2007). The list of fluorescent stains continued to expand, with alternatives like ASCQ_Ru, a ruthenium complex related to SR, as well as Rubeo, Krypton, and Flamingo, which have become more available and widely used (Tokarski et al. 2006).

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), a cornerstone in proteomics, separates proteins based on molecular weight and charge (Al-Tubuly 2000; Liu et al. 2014a) (Table 1). This technique has been widely employed in studies involving domestic animals to gain insights into their proteomes (Adnane et al. 2018b). For instance, in research aimed at identifying potential biomarkers for chronic kidney disease (CKD) in dogs, urine samples were collected and subjected to SDS-PAGE (Ferlizza et al. 2020). This separation technique allowed researchers to visualize protein differences. Notable findings included variations in uromodulin, albumin, and arginine esterase levels between healthy dogs and those with CKD. These precise quantifications offered valuable diagnostic information. Likewise, bovine cervicovaginal mucus was analysed for measurement of inflammatory biomarkers reflecting uterine inflammation, and SDS-PAGE was used to breakdown proteins of mucus and monitor proteins fractions specific for targeted biomarkers (Adnane et al. 2017, 2018a).

Gel-Based Liquid Chromatography-Mass Spectrometry (GeLC-MS), an extension of SDS-PAGE, addresses challenges posed by limited tissue samples in domestic animal studies. It combines gel electrophoresis with mass spectrometry to enable efficient protein characterization with minimal starting material (Makridakis and Vlahou 2018). This method has facilitated in-depth analyses of protein expression profiles in various domestic animal tissues, contributing to a better understanding of their biology and health. For instance, in a study focusing on oral tumours in dogs, GeLC-MS was employed to search for salivary biomarkers (Ploypetch et al. 2020). Researchers investigated the expression of proteins like protein tyrosine phosphatase non-receptor type 5 (PTPN5), along with other candidates. Results showed increased PTPN5 expression across various tumour groups compared to the control group with periodontitis (CP). Moreover, the study identified the presence of tumour protein p53 (p53) in PTPN5-related interactions. This demonstrates the potential of GeLC-MS to uncover salivary biomarkers associated with canine oral tumours.

A sophisticated variant of traditional SDS-PAGE, SDS-PAGE focusing, has emerged as a valuable tool in gel-based proteomics. In this technique SDS-protein complexes are immobilized at specific locations along the gel matrix, driven by a gradient of positive charges (Zilberstein et al. 2007a). As a result, protein separation takes on an unconventional pattern, with smaller proteins and peptides remaining near the application point, while larger proteins migrate farther down the gel. SDS-PAGE focusing offers several advantages, including accurate separation of small-size fragments and enhanced resolution of proteins with closely spaced molecular weights (Zilberstein et al. 2007b). This innovation enhances the accuracy and sensitivity of protein profiling in studies involving domestic animals.

Another gel-based proteomics have been developed aiming to improve its capabilities. For example, difference in gel electrophoresis (DIGE) allows for the comparison of two or more protein samples within the same gel, reducing gel-to-gel variation and increasing accuracy (Lottspeich 2009).

Overall, gel-based proteomics remains a valuable technique for protein separation and identification in domestic animals and has contributed to our understanding of various biological processes and disease states.

Targeted proteomics

Targeted proteomics has emerged as a robust method for the sensitive, precise, and reproducible detection and quantification of specific proteins (Aebersold et al. 2016; Borras

and Sabido 2017). Compared to broader mass spectrometry-based screening proteomics, which quantifies thousands of proteins without prior knowledge of the whole proteome composition, targeted proteomics can simultaneously quantify 10 to 100 target proteins (Borras and Sabido 2017; Picotti et al. 2013) (Table 1). Such precision is particularly valuable in the context of domestic animal research, where accurate measurement of specific biomolecules is essential.

Targeted proteomics encompasses various mass analysers, including combinations of quadrupoles, orbital ion traps (orbitrap), and time-of-flight mass analysers (Borras and Sabido 2017). Notably, after several improvements, targeted proteomics has the potential to move beyond quantifying only a few dozen selected proteins, as demonstrated by methods capable of quantifying thousands of peptides and proteins (Liu et al. 2013, 2014b). Two prominent strategies have emerged: Selected Reaction Monitoring (SRM) and Parallel Reaction Monitoring (PRM). SRM focuses on specific peptides chosen for quantifying a protein of interest (Korbakis et al. 2015). It operates on triple quadrupole mass spectrometers, isolating a precise peptide precursor ion before fragmentation and monitoring a specific fragment ion for quantification (Doerr 2013). The capacity to monitor multiple transitions within a method depends on chromatographic peak width and sampling rate, necessitating a minimum of 8 to 10 data points per chromatographic peak for accurate quantification.

Expanding the number of monitored transitions can be achieved by selectively recording transitions around the peptide's retention time, a technique sometimes referred to as Multiple Selection Monitoring (MRM) (Borras and Sabido 2017). In contrast, PRM simultaneously analyses all fragment ions of a pre-selected peptide (Peterson et al. 2012). It uses a quadrupole mass analyser to isolate the peptide ion of interest with precision, and the resulting fragment ions are concurrently screened (Borras and Sabido 2017). PRM offers several advantages, including a high dynamic range, exceptional signal-to-noise ratios, and superior sensitivity and specificity for peptide and protein quantification (Lawrence et al. 2016; Martinez-Garcia et al. 2016).

Although there are few reports on the use of targeted proteomics in domestic animals, there is potential and a need for wider application in farm animals such as dairy cattle and poultry. Here, it can revolutionise disease monitoring, nutritional assessment, and breeding strategies by allowing precise monitoring of milk protein profiles leading to increased milk quality and production (Das et al. 2022; Maity et al. 2020a). In poultry, it could help identify specific disease resistance-associated proteins, potentially leading to the development of more robust and disease-resistant breeds (Dar et al. 2018). Despite its many advantages, targeted proteomics does have limitations, including lower throughput compared to traditional enzyme-linked immunosorbent assay (ELISA) methods (Yoneyama et al. 2017). However, innovations like rapid Matrix-Assisted Laser Desorption/Ionization-MS (MALDI-MS) hold promise for overcoming this limitation, enabling high-speed protein quantification.

Mass spectrometry-based

MS-based proteomics uses MS to identify and quantify proteins in biological samples (Table 1). It can be used to study protein expression, interactions, modifications, and functions in different conditions (Han et al. 2008; Neagu et al. 2022; Noor et al. 2021) (Fig. 1). One of the applications of mass spectrometry-based proteomics is in veterinary and farm animal research, where it can help to understand animal health, disease, nutrition, reproduction, and genetics (Soares et al. 2012). For instance, a tandem mass tag (TMT)-based quantitation and mass spectrometry analysis was employed to identify and quantify bovine whey proteins at early, mid, and late lactation stages (Mol et al. 2018) (Table 2). A total of 564 proteins were detected, and among them, 403 proteins showed differential abundance at different lactation stages. The analysis revealed that 51% of the identified milk proteins possessed signal peptides, which is a typical characteristic of body fluid proteomes. These findings shed light on the dynamic changes in the whey proteome during lactation and provide insights into the biological processes underlying milk production in domestic animals.

The MALDI method is a soft ionization technique that involves the addition of a matrix to the protein sample, which absorbs the laser energy and facilitates the ionization of the proteins (Neagu et al. 2022). MALDI-MS has been used for the identification and quantification of proteins in various domestic animal species, including cattle, pigs, and poultry. MALDI can be used for animal protein analysis in various ways. One of them is MALDI imaging mass spectrometry (MALDI-MSI), which allows visualizing the spatial distribution of proteins and other molecules within thin slices of animal tissue (Reyzer et al. 2010) (Table 1). This can help reveal molecular changes associated with diseases, development, or environmental factors. Another application is MALDI-MS/MS-based proteomics, which can identify and characterize proteins and peptides from animal samples, including their post-translational modifications (PTMs) or protein-protein interactions (PPIs) (Ryan et al. 2019). This can help understand biological functions, pathways, and networks.

Electrospray ionization (ESI) is a soft ionization technique that can generate gas phase ions with multiple charges from liquid samples (Loo 2000). ESI-MS is widely used in proteomics to identify or characterize proteins and peptides, especially those with high polarity, low volatility, or

Table 2 Prominent proteomics experiments in the veterinary field. These experiments hold great promise for fundamental and applied discoveries, as well as comparative applications to human diseases

Disease/situation	Animal species	Proteomic techniques	Main findings	References
Chronic kidney disease (CKD)	Dog	SDS-PAGE to investigate kidney failure biomarkers in urine	- Variations in uromodulin, albumin, and arginine esterase levels between healthy dogs and those with CKD	(Ferlizza et al. 2020)
Trichomoniasis	Bovine	SDS-PAGE combined with ELISA to investigate the role of the third component of complement (C3) in host parasite interactions	- First time detection of C3 in genital secretion - C3 concentration increase in vaginal secretions in animals	(Kania et al. 2001)
Oral tumours	Dog	Gel-Based Liquid Chromatography-Mass Spectrometry (GeLC-MS) to search for salivary biomarkers	Increased protein tyrosine phosphatase non-receptor type 5 (PTPN5) expression across various tumour groups compared to the control group with periodontitis (CP).	(Ploypetch et al. 2020)
CKD	Dog	HPLC to measure essential and non-essential amino acids affected with	Dogs with CKD had significantly lower levels of most essential amino acids (i.e., L-histidine, L-isoleucine, L-leucine)	(Lippi et al. 2022)
dynamic changes in the whey proteome during lactation	Bovine	Tandem Mass Tag (TMT)-based quantitation and mass spectrometry to identify and quantify whey proteins in milk	403 proteins showed differential abundance at different lactation stages	(Mol et al. 2018).
canine ivermectin toxicity	Dog	ElectroSpray Ionization-Mass Spectrometry (ESI/MS) to semiquantitatively estimate concentrations of ivermectin in the tissue samples	Ivermectine was rapidly detected, and toxicity was confirmed when compared to the normal recommended concentration	(Lehner et al. 2009)
bioactive properties of bovine milk	Bovine	ElectroSpray Ionization-Mass Spectrometry (ESI/MS) to identify major polypeptides from bovine milk and their possible bioactive properties	The method is effective in identifying low molecular mass peptide sequences in food protein hydrolysates.	(Le et al. 2017; O'Keeffe and FitzGerald 2015).
growth rate and meat quality	Pig	Isobaric tag for relative and absolute quantification (iTRAQ) for proteomic analysis of the longuissimus dorsi muscle	Protein interaction network predictions identified specific proteins regulating muscle growth and muscle fiber differences while others influenced lipid deposition ability	(Wang et al. 2017)
Porcine Circovirus Type 3 (PCV3) infection	Pig	iTRAQ labeling combined with LC-MS/MS analysis to quantitatively determine the changes in cellular proteins in the lungs	- Differentially upregulated and downregulated proteins in the PCV3-infected group compared to the control group - These proteins are primarily involved in metabolic processes, innate immune responses, MHC-I and MHC-II components, and phagosome pathways.	(Jiang et al. 2020)
Calcification process of egg	Duck	TRAQ MS/MS to analyse the eggshell proteome	- Several proteins exhibit differential abundances between the inner, partially mineralized eggshell and the fully formed eggshell. - Several matrix proteins are shared among eggshells from various domesticated bird species,	(Zhu et al. 2019)
protein profiles during the preovulatory period	Bovine	iTRAQ labelling and mass spectrometry to investigate the effect of oestradiol (E2) and countify protein profiles in bovine follicular fluid (FF) and plasma (PL)	- E2 levels correlated with protein changes in both PL and FF, - These changes fall under categories such as cellular and metabolic processes.	(Afedi et al. 2021)
Milk production mechanisms	Bovine	iTRAQ combined with RNA seq to identify potential biomarkers for milk quality and quantity	Genes and proteins that exhibited differential expression were primarily associated with lipid metabolism, the size and growth of the mammary gland, and energy metabolism.	(Dai et al. 2018)
Assessing pain during routine surgical procedures in farm animals	Bovine	Sequential Windowed Acquisition of All Theoretical Mass Spectra (SWATH-MS) to detect protein biomarkers associated with pain and inflammation	Several candidate biomarkers were detected and linked to stress, wound healing, immune responses, blood coagulation, and inflammatory and acute phase responses,	(Ghodasara et al. 2022)
Septicemia/toxemia (septox)	Chicken	Label-free spectral counting to detect septox in liver	100% correlation with histopathological diagnosis	(Dey et al. 2003)

Table 2 (continued)

Disease/situation	Animal species	Proteomic techniques	Main findings	References
Normal and overconditioned body conditions (BCS) during the transition period	Bovine	Bioinformatics analyses in conjunction with proteomics to investigate changes in the plasma proteome during the transition period	Differentially abundant proteins related to acute inflammatory response, complement activation regulation, protein activation cascade, and humoral immune response as significantly enriched in the Low-BCS group	(Ghaffari et al. 2020)
Rabies prevention and postvaccination monitoring	Dog	Enzyme-linked immunosorbent assay (ELISA) to measure rabies-specific antibody titres in serum.	High sensitivity, specificity, and accuracy rates	(Fitria et al. 2023)
SARS-CoV-2	Several animal species (experimental, domestic, companion and wild animals)	ELISA to detect SARS-CoV-2-specific antibodies	- No positive testing - Animals were not exposed to or infected by SARS-CoV-2	(Deng et al. 2020).
Mastitis	Bovine	Multi-omic approaches to investigate the metagenomics of pathogens in milk	Accurate detection of various bacterial genera	(Kusumawati et al. 2021)
intramuscular fat development	Bovine	Proteotranscriptomics to identify differentially expressed genes in skeletal muscle	Genes predominantly expressed in longuissimus dorsi from Japanese Black cattle are associated with unsaturated fatty acid synthesis, fat deposition, and thyroid hormone pathways.	(Wang et al. 2005)

thermal instability (Loo 2000). ESI-MS can be combined with other techniques such as chromatography, isoelectric focusing, or tandem mass spectrometry to achieve higher resolution, sensitivity, and specificity (Doerr 2013; Xu and Sun 2021). ESI-MS can also be applied to drug discovery, biomarker detection, and protein-protein interaction analysis (Zubair 2021). For example, ESI-MS has been used to confirm canine ivermectin toxicity by estimated semiquantitative concentrations of ivermectin in the tissue samples of two died dogs (Lehner et al. 2009). This method seems to be valuable for rapid detection and confirmation of toxic exposures in veterinary diagnostics, aiding in both antemortem clinical cases and postmortem forensic investigations. ESI-MS has also been used to identify low molecular mass peptides (BAPs) from bovine milk and their possible bioactive properties (O'Keeffe and FitzGerald 2015). The technique was revealed to be effective in identifying low molecular mass peptide sequences in food protein hydrolysates.

Chromatography-based methods are widely used in combination with MS in proteomics research for the separation and purification of complex protein mixtures (Shi et al. 2004). These methods utilize the physicochemical properties of proteins such as size, charge, and hydrophobicity to separate them into individual components (Fig. 1). Various types of chromatography techniques, including liquid chromatography (LC) (Shi et al. 2004), ion exchange chromatography (IEC) (Cummins et al. 2017), size exclusion chromatography (SEC) (Tiambeng et al. 2022), and hydrophobic interaction chromatography (HIC) (Mahn 2012), have been developed and are routinely used in proteomics studies. These methods are highly sensitive and can detect low-abundance proteins, making them valuable tools in the identification and quantification of proteins in complex biological samples.

For instance, HPLC was used to measure essential and non-essential amino acids in dogs affected with chronic kidney disease (CKD) at different stage and compared them with healthy control dogs (Lippi et al. 2022) (Table 2). The results showed that dogs with CKD had significantly lower levels of most essential amino acids (i.e., L-histidine, L-isoleucine, L-leucine) and some non-essential amino acids (i.e., L-glutamic acid and glycine) than healthy control dogs. Some amino acids were also correlated with markers of calcium–phosphate metabolism (cysteine), metabolic acidosis (phenylalanine and leucine), and protein-energy wasting syndrome (PEW) (Lippi et al. 2022). The study suggested that dogs with CKD have altered serum amino acid profile that may be related to their clinical status and complications.

Label-based and label-free approaches in protein identification

Because proteomics aims to determine changes in protein abundance between different biological states or conditions (Mol et al. 2018; Steen and Pandey 2002; Zimmermann et

al. 2021) (Fig. 1), there are two main approaches to quantitative proteomics: label-based and label-free (Terzi and Cambridge 2017; Zimmermann et al. 2021) (Tables 1 and 2).

Label-based approaches involve the use of stable isotope labelling, such as isobaric tags for relative and absolute quantitation (iTRAQ) or TMT, to label proteins in two or more samples with different isotopes (Wang et al. 2017). The labelled peptides are then combined and analysed using MS. The ratio of the labelled peptides is used to determine the relative abundance of the proteins in the different samples. iTRAQ-based proteomic approach has been used in domestic animals to study various biological processes, such as muscle growth, lipid deposition, eggshell formation, follicular development, and viral infection (Afedi et al. 2021; Jiang et al. 2020; Wang et al. 2017; Zhu et al. 2019).

iTRAQ proteomics, combined with RNA seq, were employed to comprehensively investigate the differences in molecular pathways and biological processes in the mammary tissues of lactating cows, with respect to milk quality and quantity (Dai et al. 2018). The results indicated that differentially expressed genes and proteins were mainly involved in lipid metabolism, mammary gland size and growth and energy metabolism.

Label-based approaches are highly reproducible and sensitive but can be expensive and time-consuming. Label-free approaches do not involve the use of stable isotope labeling. Instead, the abundance of peptides is measured based on their signal intensity in the mass spectrometer (Noor et al. 2019). Some examples of label-free quantitative proteomic techniques that have been applied to domestic animals are Data Independent Acquisition (DIA), Sequential Windowed Acquisition of All Theoretical Mass Spectra (SWATH-MS), Spectra.

DIA uses MS to fragment proteins and generate a spectral library that can be used to quantify protein expression levels in subsequent samples (Doerr 2014). DIA can be extensively used for the analysis of drug-metabolizing enzymes and transporter that help to ease drug discoveries and drug-effect investigation (Li et al. 2021). In addition, DIA has been used in human to analyse hydrophilic metabolites and lipids in papillary thyroid carcinoma (TC) serum samples (Zhou et al. 2017). This study proposes a novel metabolomic data acquisition workflow that combines data-dependent acquisition (DDA) and DIA analyses to achieve better metabolomic data quality, including enhanced metabolome coverage, tandem mass spectrometry (MS 2) coverage, and MS 2 quality. This workflow is named data-dependent-assisted data-independent acquisition (DaDIA). While no data has been found regarding the veterinary field, it would be interesting to see if similar workflows in studying the proteomes of domestic animals.

SWATH-MS is a variation of DIA that allows for the comprehensive quantification of proteins in complex samples (Frederick and Ciborowski 2016; Ludwig et al. 2018). The principle of SWATH-MS technology involves employing a TripleTOF or Orbitrap mass spectrometer, and SWATH data acquisition is typically conducted by a single precursor ion (MS1) scan coupled with product ion (MS2) scans of multiple precursor isolation windows (Chen et al. 2021b). SWATH-MS has been used to quantify protein in plasma using chromatography (van der Laan et al. 2020). The latter study discusses how DIA methods, including the SWATH approach are being increasingly used in metabolomics by enabling simultaneous metabolite identification and quantification through the acquisition of MS/MS spectra for all analytes in a single run.

Recently, SWATH-MS was used to detect protein biomarkers associated with pain and inflammatory processes associated with routine surgical procedures in cattle (Ghodasara et al. 2022). Several candidate biomarkers were detected and linked to stress, wound healing, immune responses, blood coagulation, and inflammatory and acute phase responses, providing a more objective means to assess pain and inflammation resulting from tissue injury. In addition, SWAT-HS is applicable in different field related to biomarker studies (Muntel et al. 2015), genetic studies (Okada et al. 2016) and drug studies (Litichevskiy et al. 2018). While label-free approaches are faster and less expensive than label-based approaches they can be less accurate due to technical variability.

Spectral counting is a widely used label-free quantitative proteomic method that relies on counting the number of MS/MS spectra generated for each protein in a sample to estimate their abundance (Fermin et al. 2011). Proteins are identified by comparing the experimental spectra to a reference database, using search algorithms such as SEQUEST or Mascot (Liu et al. 2004). This technique has been applied in various biological and medical research studies (Lundgren et al. 2010). For example, spectral counting was employed to investigate the proteome of chicken liver tissue in case of septicaemia (Dey et al. 2003). The technique is simple and high-throughput capable, which make it a useful tool for large-scale proteomic studies. However, the accuracy of spectral counting can be affected by several factors such as non-reproducibility of results, peptide detectability, incomplete protein digestion, and sample complexity (Zhang et al. 2006).

Functional proteomics is a branch of proteomics that focuses on understanding the functions of proteins within a biological system (Monti et al. 2007). This includes identifying protein-protein interactions, post-translational modifications, and the localization of proteins within cells or tissues (Monti et al. 2005) (Fig. 1). In addition, it involves

the interaction of proteins with other biomolecules such as nucleic acids, lipids, and carbohydrates. The overall objective of functional proteomic is to gain a better understanding of the biological processes and pathways that are involved in various cellular functions and diseases in different species, including domestic animals. There are several techniques used in functional proteomics, including protein microarrays, protein-protein interaction assays, and imaging mass spectrometry (Kustatscher et al. 2022).

Protein-protein interaction assays based on mass spectrometry are highly used in advanced proteomic analysis (Kustatscher et al. 2022). Several techniques are used to conduct such extensive study, among which crosslinking MS (O'Reilly and Rappsilber 2018), affinity purification MS (Smits and Vermeulen 2016), co-fractionation MS (Lundberg and Borner 2019), proximity labelling MS (Gingras et al. 2019), antibody-based proteomics (Thul et al. 2017) and protein co-regulation (Kustatscher et al. 2019) are the most investigated.

Protein identification and bioinformatics

Bioinformatics tools play a critical role in proteomics research by enabling the processing, analysis, and interpretation of large proteomics datasets (Fig. 1). These tools can help researchers identify and quantify proteins, analyse protein-protein interactions, identify post-translational modifications, and perform functional analysis of proteomics data (Gligorijevic et al. 2018).

One of the main challenges in proteomics is the identification and quantification of proteins from complex mixtures. This can be achieved using various bioinformatics tools, such as search engines that match the mass spectra data to protein sequence databases (Fadda and Almeida 2015). Several tools and software for protein quantification are available and include MaxQuant (www.maxquant.org), Proteome Discoverer (www.thermofisher.com), and Scaffold (https://support.proteomesoftware.com/hc/en-us). In addition, several online databases are available for storing and sharing proteomics data, such as ProteomeXchange (www.proteomexchange.org) and Global Proteome Machine Database (GPMDB) (https://thegpm.org/). These databases enable the integration of proteomics data from different sources and facilitate the analysis and interpretation of proteomics data.

A study investigated changes in the plasma proteome of normal and obese dairy cows during the transition period was conducted using a combination of quantitative proteomics and bioinformatic analysis (Ghaffari et al. 2020). The results showed that high body condition scores around calving were associated with alterations in protein pathways related to acute inflammatory response and regulation of complement and coagulation cascades in transition cows.

Antibody-based approaches for validating regulated proteins

Antibody-based proteomic methods hold a pivotal role in conducting high-throughput, multiplexed assessments of protein regulation in various health and disease scenarios. These innovative approaches offer compact configurations that allow the profiling of specific proteins with precision, sensitivity, and speed (Brennan et al. 2010). This capability extends to capturing proteins across a broad abundance range, encompassing even those with low levels of occurrence, within intricate proteomic landscapes such as serum (Wingren 2016).

Antibody-based methods such as western blotting are widely used in proteomics to detect and quantify specific proteins in complex biological samples (Fig. 1). This involves the transfer of proteins from a gel onto a membrane, followed by incubation with a primary antibody specific to the target protein, and detection with a secondary antibody conjugated to a detection reagent (Barkovits et al. 2021; Ohara et al. 2006). The protocol turns to be rather lengthy and relies on the availability of specific antibodies. A recent study describes a method called "rapid western blot" that can reduce the time and cost of western blot analysis for protein quantification (Barkovits et al. 2021). A fluorescent dye is used to label proteins before transferring them to a membrane. The membrane is then scanned by a fluorescence scanner to obtain an image of the protein bands. The image can be used to measure the relative abundance of target proteins by comparing their fluorescence intensity with that of a reference protein. The method also allows for multiplexing, which means detecting more than one protein at a time using different fluorescent dyes. The latter study demonstrates the applicability and reliability of the method by comparing it with conventional Western blot analysis using different types of samples, such as cell lysates, plasma, and urine (Barkovits et al. 2021). The results show that the method can achieve similar or better accuracy and precision than conventional Western blot analysis, while saving time and resources.

Another antibody-based method is enzyme-linked immunosorbent assay (ELISA), which is based on the binding of an antibody to a target protein immobilized on a solid surface, followed by detection with an enzyme-conjugated secondary antibody (Lequin 2005) (Tables 1 and 2). ELISA has been used for the detection of a wide range of proteins in various biological fluids, including blood, milk, and urine, in domestic animals (Adnane et al. 2018b; Biancifiori and Cardaras 1983; Cronin et al. 2015; Esposito et al. 2020). For

instance, antirabies antibodies were detected using ELISA technique (Fitria et al. 2023). This study evaluated an in-house ELISA (BukTi-Vet) for detecting rabies-specific IgG antibodies in domestic dogs. The performance of BukTi-Vet was compared with two commercial ELISA kits (Platelia II and Pusvetma) using serum samples from dogs vaccinated with different rabies vaccines. As major results, BukTi-Vet had high sensitivity, specificity, and accuracy, and was excellent for use as a confirmatory and screening test for rabies antibodies. Also, BukTi-Vet had good agreement with Platelia II and Pusvetma and could be used as an alternative method for post-vaccination monitoring in dogs.

Likewise, ELISA was used to detect SARS-CoV-2-specific antibodies in 1,914 serum samples from 35 animal species, including experimental, domestic, companion and wild animals (Deng et al. 2020). None of the samples tested positive for SARS-CoV-2 antibodies, suggesting that these animals were not exposed to or infected by SARS-CoV-2. Also, ELISA was applied to detect epizootic haemorrhagic disease virus (EHDV) antibodies in 2,199 serum samples from cattle, sheep, goats, wild deer and zoo animals (Breard et al. 2020). They found that this ELISA kit was specific and sensitive for detecting EHDV antibodies in domestic and wild ruminants.

Aiming to understand the process of uterine inflammation in postpartum dairy cows, cytokines, chemokines, and acute phases proteins were successfully measured in vaginal mucus using ELISA (Adnane et al. 2017; Adnane et al. 2018a). The technique allowed the identification of biomarkers that can be used to predict the uterine disease before the occurrence of clinical symptoms (Adnane et al. 2017).

Despite the advantages of antibody-based methods, they also have some limitations, such as the need for specific antibodies and the potential for cross-reactivity and non-specific binding (Barkovits et al. 2021). Therefore, it is essential to validate the specificity and sensitivity of antibody-based methods before applying them to proteomics studies.

Sample pre-treatment for enhanced proteomic analysis

Sample pre-treatment is a critical step in proteomic analysis that plays a significant role in improving sensitivity, coverage, and the overall quality of results (Fig. 1). This technique involves the separation of peptides based on their distinct physical or chemical properties, including size, charge, hydrophobicity, or their affinity to specific ligands. By reducing the complexity and dynamic range of peptide mixtures, pre-fractionation methods enhance the proteomic analysis process.

Sub-cellular fractionation

It focuses on isolating various organelles or subcellular compartments from cells or tissues (Walker 2002). This technique is particularly valuable when studying proteins associated with specific cellular locations, such as nuclei, mitochondria, membrane proteins, or cytosolic proteins (de Araujo and Huber 2007). Sub-cellular fractionation can be achieved by employing different detergents, buffers, centrifugation steps, or commercially available kits. This approach not only simplifies the proteomic analysis but also allows for the exploration of distinct subcellular proteomes.

Enrichment of phospho/glycopeptides

Post-translational modifications like phosphorylation and glycosylation are vital regulators of protein function and interactions. To enrich peptides bearing these modifications, methods such as Metal Oxide Affinity Chromatography (MOAC) and titanium dioxide (TiO2) (Chen et al. 2021a; Chu et al. 2022), Immobilized Metal Affinity Chromatography (IMAC) (Zheng et al. 2020), zirconium dioxide (ZrO2) (He et al. 2022), lectins, or antibodies are employed. This selective enrichment aids in uncovering critical insights into the role of modified peptides in cellular processes.

Reversed phase chromatography

Peptide separation based on hydrophobicity is achieved through reversed phase chromatography. This method employs a gradient of organic solvents on a column with a hydrophobic stationary phase (Niu et al. 2022). The pH can be adjusted to achieve varying selectivity and resolution levels. This versatile technique is valuable for examining peptides with different hydrophobic properties and allows for tailored separation conditions.

Ion-exchange chromatography

Separation based on charge is accomplished using ion-exchange chromatography, which employs a column with a charged stationary phase and a buffer with variable salt concentration or pH (Ngere et al. 2023). Depending on the type of charge on the column, this method can be cation-exchange (CEX) or anion-exchange (AEX) chromatography. This approach enables the precise isolation of peptides characterized by distinct charge properties, thereby offering valuable insights into the functional significance of charge-modified peptides.

Size-exclusion chromatography

Size-exclusion chromatography is another powerful pre-fractionation technique. It separates peptides primarily based on their size using a column with porous stationary phase that excludes larger molecules from entering the pores (Striegel 2022). This method can also distinguish peptides based on their shape or conformation. Size-exclusion chromatography offers a unique perspective on the proteome by focusing on the structural attributes of peptides.

Incorporating these pre-fractionation techniques into proteomic workflows enhances the depth and accuracy of analyses, enabling researchers to delve deeper into the complexities of the proteome. Whether isolating specific subcellular components, enriching modified peptides, or fine-tuning separation based on physical properties, these methods are indispensable tools for advancing our understanding of protein function and interactions.

Applications of proteomics in domestic animals with an emphasis on the tropics and the subtropics

Monitoring animal health and welfare through biomarkers is crucial for understanding their physiological processes and adaptation to environmental conditions (de Almeida et al. 2019) (Table 2).

Proteomics and livestock production

Proteomics has emerged as a powerful tool for improving livestock production by identifying key proteins and pathways involved in various aspects of animal health, growth, and reproduction.

Improving animal growth and performance

Proteomics is ideal for studying biological processes such as muscle development which underlies growth, an economically important trait for meat animal production. For instance, in pigs, proteins involved in structure and function of muscle fibre were identified (Kim et al. 2018). Likewise, tolerance to seasonal weight loss (SWL) in tropical sheep breeds was described in sheep fed varying diets under tropical conditions (Ferreira et al. 2017). Herein, a label-free proteomics approach was used to identify 668 proteins, with 95 showing differential expression in different breeds (Ferreira et al. 2017). Six putative markers for restricted nutritional conditions were suggested, as well as two potential markers related to SWL tolerance (Ferreira et al. 2017).

Furthermore, utilizing label-free proteomics to analyse hepatic tissue from Damara and the Merino sheep breeds in relation to SWL (Miller et al. 2019a, b). Distinct fatty acid metabolic pathways were detected in the two breeds. Proteins associated with fatty acid metabolism are found to be more abundant in the Damara, potentially serving as markers of tolerance to nutritional stress. Thus, proteomics offers valuable insights for developing sustainable long-term solutions for farmers facing SWL-related production issues, particularly in drought-prone regions of the tropics and the Mediterranean.

A study investigated changes in the plasma proteome of normal and over conditioned dairy cows during the transition period was conducted using a combination of quantitative proteomics and bioinformatic analysis (Ghaffari et al. 2020). The results showed that over conditioning around calving was associated with alterations in protein pathways related to acute inflammatory response and regulation of complement and coagulation cascades in transition cows.

Enhancing feed efficiency and nutrient utilization

By identifying key proteins and pathways involved in nutrient partitioning, proteomics provides markers for improving feed efficiency and reducing the environmental impact of animal production. Proteomics analyses showed that branched-chain amino acids (BCAA) degradation and enhanced mitochondrial oxidative metabolism were activated in muscle due to leucine and threonine supplementation in Holstein cattle (Yu et al. 2020). The leucine-threonine group showed greater BCAA availability and mitochondrial oxidative activity. The potential allergenicity of *Tenebrio molitor* (mealworm) proteins as an alternative source of dietary protein was investigated (Premrov Bajuk et al. 2021). Abundant skeletal muscle sarcoplasmic proteins were reported in pigs exposed to heat stress for 12 h (Cruzen et al. 2015). The increased proteins function in glycolysis, glycogenesis, and glycogenolysis suggesting enhanced glycolytic capacity in response to heat stress. An antioxidant response to heat stress was also observed, as manganese superoxide dismutase abundance increased and peroxiredoxin 2 decreased (Cruzen et al. 2015).

Improving meat quality and safety

Gene ontology and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analyses can also be used to improve the quality and safety of meat products by identifying proteins and pathways associated with meat tenderness, marbling and flavour (Bouley et al. 2004). A protein reference map of the semitendinosus muscle was constructed where 129 protein spots corresponding to 75 different gene

products were identified (Almeida et al. 2015). Most of these proteins are involved in metabolism, cell structure, cell defence, and contractile apparatus. This study provided deeper understanding the mechanisms controlling postmortem muscle metabolism and meat quality.

Likewise, beef (tenderloin and flank) from Simmental cattle contains 17 amino acids with significant differences in abundance between steak types, while 128 differentially expressed proteins (DEPs) (44 up-regulated, 84 down-regulated), according to a proteomic analysis using iTRAQ approach (Lei et al. 2020).

Proteomics of veterinary medicine

Biomarker discovery and disease diagnosis

Proteomics has shown promise in the identification of biomarkers for the diagnosis and monitoring of diseases in domestic animals. By analysing the protein profiles of bodily fluids or tissues, proteomics can identify specific proteins or protein patterns that are associated with diseases.

For example, Surface Enhanced Laser Desorption Ionization Time-of-Flight Mass Spectrometry (SELDI TOF-MS) was used to investigate paratuberculosis, a chronic granulomatous enteritis in sheep serum (Zhong et al. 2011). Multivariate biomarker models were developed to differentiate sheep with paratuberculosis and vaccinated-exposed sheep from unexposed animals. SELDI was also used to monitor protein profile changes over time during an experimental infection trial, revealing consistent proteomic features associated with exposure to *Mycobacterium tuberculosis*. Two putative serum biomarkers, transthyretin, and alpha haemoglobin are abundant acute phase reactants in the serum proteome of bovid (Zhong et al. 2011).

Chronic kidney disease (CKD) in dogs is a progressive and irreversible condition, but current biomarkers are lacking. Proteomic analysis using mass spectrometry in urine samples from healthy dogs and dogs with spontaneous CKD was conducted aiming to characterize the urine proteome and metabolome and identifying potential biomarkers (Ferlizza et al. 2020). Uromodulin and albumin concentrations were significantly altered in CKD dogs compared to healthy animals, with a more complex protein pattern indicating mixed glomerular and tubular proteinuria. Metabolomic analysis using NMR spectra identified 86 metabolites in healthy dogs, belonging to various pathways. Seventeen metabolites showed significantly different concentrations in CKD dogs, including carnosine, trigonelline, and cis-aconitate, which could be potential biomarkers for CKD in dogs.

Another study characterised protein composition of synovial fluid (SF) in health and osteoarthritis (OA) using liquid chromatography mass spectrometry (LC-MS/MS) and protein equalization method (Peffers et al. 2015). A total of 764 proteins were identified in SF, with 10 proteins showing differential expression in OA SF. S100-A10, a calcium binding protein, was upregulated in OA. Several new OA-specific peptide fragments (neopeptides) were also identified. The study provides insights into the SF proteome in OA and identifies potential biomarkers for distinguishing between normal and OA joints.

Pharmacology and drug discovery

Proteomics has also shown promise in the development of new drugs and the improvement of existing drugs for the treatment of diseases in domestic animals (Aslam et al. 2017). By analysing the proteome of cells or tissues, proteomics can identify potential drug targets according to the pathways that are involved in disease pathogenesis (Jeffery et al. 2005; Lill et al. 2021).

For example, a study using proteomics to analyse the proteome of canine osteosarcoma cells identified several potential drug targets, including proteins involved in cell proliferation and survival (Weinman et al. 2021). The study found that exosomes from carboplatin-resistant canine osteosarcoma cell lines effectively transferred drug-resistance to sensitive cells and were shown to exhibit a unique cargo that correlates with their resistance profile.

Reproduction and cyclicity

Proteomic analysis of reproductive tissues, such as ovaries, testes, uterus, and placenta. has led to the identification of numerous proteins that play critical roles in reproductive processes. For instance, follicular fluid proteins in bovine carrying a high ovulation rate allele were analysed and compared with those who did not have the allele (Kamalludin et al. 2018). Two proteins, glia-derived nexin precursor (SERPINE2) and inhibin β B chain precursor (INHBB), were found to be differentially expressed between carriers and non-carriers of the Trio allele for high ovulation rate. The reduction in expression of these proteins in carrier animals is consistent with the inhibitory effect of SMAD family member 6 (SMAD6), which is overexpressed in carrier females, on the transforming growth factor-β–bone morphogenetic protein signalling pathway.

Changes in the protein expression of spermatozoa during liquid storage can be investigated using comparative proteomics. Semen from boars was analysed before and after three days of liquid preservation and sperm parameters were evaluated, and protein profiles were compared using two-dimensional SDS-PAGE and mass spectrometry (Premrov Bajuk et al. 2020). Four protein spots were found to be significantly different between fresh and stored sperm,

including ATP citrate lyase, chaperonin TCP1 subunit ε, probable phospholipid-transporting ATP-ase, and cytosolic non-specific dipeptidase. These proteins could potentially serve as biomarkers for evaluating boar semen quality and sperm survival after liquid storage.

Understanding disease mechanisms and pathophysiology

Theileria annulata (*T. annulata*), an apicomplexan parasite infects myeloid cells thereby inducing dysregulated division and altered phenotype in cattle (Dobbelaere and Rottenberg 2003). TashAT are parasite polypeptides located in the host cell nucleus. They are modulators of host cell phenotype, whose expression differs in a bovine macrophage cell line transfected with TashAT2, inducing continuous cell division (Oura et al. 2006). A unique gene *bUBP43* and its ubiquitin-like substrate, *bISG15*, were expressed at low levels in *T. annulata*-infected cells (Oura et al. 2006). Thus, proteomic analysis such as 2-DE revealed the parasite protein of T. annulate implicated in hots cell phenotype changes, which for long time was unknown.

Likewise, potential biomarkers for rapid, non-invasive, and sensitive detection of sub-clinical mastitis (SCM) in dairy cows were detected using high-resolution mass spectrometry-based quantitative proteomics (Bathla et al. 2020). The analysis identified hundreds of differentially expressed proteins between normal and infected samples. The specific proteins correlated with the production of cytokines and inflammatory molecules were remarkably overexpressed. These potential biomarkers may be used to segregate affected cattle from the normal herd and support mitigation measures for prevention of SCM and CM.

TMT proteomics allowed the investigation of changes in the milk and serum proteomes of dairy cows affected by subclinical and clinical mastitis (Turk et al. 2021). A total of 237 proteins in milk and 117 proteins in serum were found to significantly change in abundance during mastitis. Interestingly, some proteins exhibited reciprocal changes between milk and serum, indicating their role in host defense and homeostasis during the disease.

Because of the economic importance of mastitis, disease pathogeny was deeply investigated in dairy cattle, using proteomic technologies. MS was performed to identify proteins that mark the transition from subclinical to clinical mastitis in dairy cows and buffaloes caused by *Staphylococcus aureus (*Maity et al. 2020b). A total of 1479 proteins were identified, among which 128 and 163 proteins were found to indicate disease progression in cows and buffaloes, respectively.

All the above studies provide valuable insights about how important to implicate proteomic analysis in detecting candidate biomarkers of several diseases, including those with high economic impact.

Emerging trends and future directions

Multi-omics integration and systems biology approaches

Integrating data from multiple "omics" platforms can provide a more comprehensive understanding of biological processes and pathways, allowing for more accurate predictions and targeted interventions (Conesa and Beck 2019). Multi-omics approaches, such as metagenomics, metatranscriptomics, metaproteomics, metabolomics, and lipidomics, can provide a mechanistic understanding of how the milk microbiome may modulate host physiology and investigates local breeds or other species in order to improve genetic potential of farm animals (Heintz-Buschart and Westerhuis 2022; Kusumawati et al. 2021; Thomas et al. 2016). The integration of multiple omics technologies can lead to a better functional understanding of the milk microbiome and its role in mastitis, aiding the development of more effective strategies for its prevention and management (Couvillion et al. 2023; Kusumawati et al. 2021).

The effects of low-dose antibiotics (LDA) on hepatocellular functions, weight gain, and metabolic imbalance in weaned piglets were investigated using multi-omics analysis (Hu et al. 2020). The experiment showed that LDA had a growth-promoting effect, as evidenced by increased body weight and average daily gain.

Epigenetic changes in gene methylation patterns were observed using liquid hybridization capture-based bisulfite sequencing (LHC-BS), with most differential methylation regions (DMRs) showing hypermethylation in the LDA group. Several DMRs were found to be enriched in genes related to fatty acid metabolism. Transcriptome analysis of liver tissues revealed differentially expressed genes (DEGs) involved in lipid metabolism and immunity, with elevated expression in the LDA group (Hu et al. 2020). Lipidome analysis of serum identified significantly differential lipids, mostly downregulated in the LDA group. These findings highlight the importance of multi-omics analyses and suggest that LDA can induce epigenetic and transcriptional changes in key genes, leading to enhanced lipid metabolism in the liver.

Single-cell proteomics and spatial proteomics

Traditionally, proteomics has relied on analysing the average protein expression levels across a bulk population of cells. However, this approach masks the heterogeneity and

complexity of individual cells, which can have different protein expression profiles and functions even within the same tissue or organ (Bagger and Probst 2020). Single-cell proteomics aims to overcome this limitation by analysing the proteome of individual cells, allowing for a more detailed understanding of cellular heterogeneity and function.

A new technology called single-cell Deep Visual Proteomics (scDVP), combines high-content imaging, laser microdissection, and multiplexed MS was developed to analyse the spatial proteome of murine hepatocytes (Rosenberger et al. 2022). scDVP identified around 1,700 proteins from a single-cell slice, revealing that half of the proteome was differentially regulated in a spatial manner, particularly near the central vein. In addition, machine learning was applied to proteome classes and images to infer the spatial proteome from imaging data alone. Interestingly, scDVP can be used in healthy and diseased animal's tissues and complements other spatial proteomics or omics technologies.

In the realm of single-cell proteomics, MS-based techniques have demonstrated notable achievements. Leveraging sophisticated automated sample preparation procedures and the integration of label-free or multiplexed data acquisition on exquisitely sensitive instruments, investigators are now consistently capable of identifying and quantifying between 1,000 and 1,500 proteins within individual cells. Nonetheless, these figures stand in stark contrast to the comprehensive collection of distinct proteins and proteoforms inherent within a cell's intricate composition.

Within the context of dairy cows and mares, the application of this technology holds significant promise for investigating distinct modifications in phagocytic activity, particularly within neutrophils, when confronted with uterine diseases (Asbury et al. 1984; Kania et al. 2001). Employing single-cell techniques would illuminate protein-level fluctuations in neutrophil function between healthy and endometritis cattle, thereby unravelling potential therapeutic targets.

Spatial proteomics is another emerging area of proteomics that allows for the analysis of protein expression in specific regions of tissue or cells. Spatial proteomics techniques, such as imaging MS and proximity-based labelling, allow for the visualization and quantification of proteins within specific regions of cells and tissues (Codeluppi et al. 2018).

Recently, spatial multi-omics technologies have emerged, allowing for the study of transcriptomes, proteomes, and metabolomes in the context of tumour-immune microenvironments in various types of cancer (Rosenberger et al. 2022). These spatial omics methods are combined with immunohistochemistry and multiparameter analysis to identify markers of cancer progression. The integration of laser capture microdissection (LCM) with nanolitre sample-preparation techniques, such as nanoPOTS, has facilitated the direct examination of individual cells, including neurons (Cong et al. 2020). Additionally, LCM has the capability to traverse tissues systematically, generating spatial proteome maps through unbiased single-cell mass spectrometry (scMS) analysis (Zhu et al. 2018).

To the best of our knowledge, there is no available study on the application of spatial proteomics specifically to the veterinary field. This emerging technology presents however an exciting opportunity for future research in veterinary science, offering novel insights into spatially resolved protein expression patterns within tissues and organs, which could have significant implications for understanding diseases and developing targeted interventions in animal health. For instance, these advanced technologies would be of great benefit to the dairy industry by creating a proteome map about the metabolism and milk yielding mechanisms in dairy cows. The emergence of single-cell proteomics and spatial proteomics represents exciting new avenues for research in proteomics in domestic animals, enabling the analysis of cellular heterogeneity and function in unprecedented detail.

Proteogenomics and proteotranscriptomics

Proteomics and genomics/transcriptomics are two complementary fields of study that provide valuable insights into the functional roles of genes and proteins in domestic animals. Proteogenomics and proteotranscriptomics aim to integrate these two fields by combining proteomics data with genomic or transcriptomic data, respectively (Fancello and Burger 2022). Proteogenomics involves the use of proteomics data to annotate and validate gene models, detect novel protein-coding genes, and identify genomic variations that result in protein sequence alterations (Menschaert and Fenyo 2017).

For example, proteogenomic studies have been used to investigate the molecular mechanisms underlying disease resistance or susceptibility in livestock, such as identifying proteins associated with immune response, inflammation, and stress response. A recent study used proteogenomics to identify unreported antimicrobial resistance genes (ARGs) in watering troughs (Dhindwal et al. 2023). As major findings, an α/β-hydrolase enzyme confirmed to inactivate macrolide. This enzyme is serine-dependent and co-occurs with emerging antibiotic resistance genes (ARGs) in various environments, including animal microbiomes and pathogens. This suggests the potential role of this enzyme in the spread of macrolide resistance and highlight the importance of understanding the mechanisms underlying antibiotic resistance in different settings.

With advancements in high-throughput sequencing, MS, and bioinformatics, proteotranscriptomics has become a valuable tool for investigating the molecular mechanisms underlying various physiological and pathological processes in domestic animals. For example, transcriptomic analysis was used to identify differentially expressed genes in skeletal muscle tissue from two breeds of cattle with different meat quality traits (Wang et al. 2005). The study used a cDNA microarray to investigate gene expression differences between the longissimus dorsi (LD) muscle of Japanese Black (JB) and Holstein (HOL) cattle. The differentially expressed genes in JB were associated with unsaturated fatty acid synthesis, fat deposition, and the thyroid hormone pathway, which aligns with the observed higher amounts of monounsaturated fatty acids in JB muscle. These finding have several applications, as they may contribute to a better understanding of the regulatory pathways involved in the development of intramuscular adipose tissue by identifying previously uncharacterized genes that are differentially regulated in different species and breed.

Artificial intelligence (AI) in veterinary proteomics

The utilization of AI and machine learning (ML) in the analysis and interpretation of complex proteomic datasets holds immense promise for advancing veterinary science. AI and ML algorithms can play a pivotal role in data preprocessing and quality control. They can automatically detect and correct errors, such as baseline shifts or noise in mass spectrometry data. ML models can identify and remove low-quality spectra, improving the overall data quality before downstream analysis.

Furthermore, proteomic datasets often contain a multitude of features, such as peptide identifications and spectral counts. AI algorithms can assist in feature selection by identifying the most informative variables for a given research question. This reduces data dimensionality and enhances the accuracy of subsequent analyses. Likewise, AI and ML excel at recognizing intricate patterns within proteomic data. They can identify clusters of proteins with similar expression profiles, which may correspond to specific biological processes or disease states. This is particularly valuable for understanding the heterogeneity of responses in veterinary contexts.

For instance, DIABLO (Data Integration Analysis for Biomarker discovery using Latent cOmponents), was proposed as a powerful multi-omics integration method designed to identify common information across diverse data types while distinguishing between different phenotypic groups (Singh et al. 2019). Through comprehensive simulations and benchmark studies involving multi-omics datasets, DIABLO demonstrates its ability to uncover molecular features of exceptional biological relevance, surpassing existing unsupervised integration techniques. Notably, DIABLO achieves predictive performance on par with state-of-the-art supervised approaches. Moreover, its versatility allows for modular-based analyses and supports cross-over study designs. In two real-world case studies, DIABLO successfully identifies a range of multi-omics biomarkers, encompassing mRNAs, miRNAs, CpGs, proteins, and metabolites, both confirming known markers and revealing novel ones. This highlights DIABLO as a promising tool for multi-omics research and biomarker discovery.

A recent study examined the impact of Seasonal Weight Loss (SWL) on Majorera and Palmera dairy goat breeds (Ribeiro et al. 2023). Using Omics tools and DIABLO, the research revealed that unrestricted goats show increased protein synthesis and pathways related to arginine catabolism and adipogenesis, reflecting their higher milk production. In contrast, restricted goats prioritize acetyl-CoA synthesis, potentially for enhanced fatty acid production or alternative energy sources. These findings shed light on how SWL affects dairy goat mammary glands and offer potential biomarkers for selection programs, addressing SWL's impact on animal production in tropical and Mediterranean regions.

Also, AI techniques can build predictive models based on proteomic data. For instance, they can develop algorithms that forecast disease progression or treatment outcomes in individual animals. This personalized approach has the potential to revolutionize veterinary medicine, allowing for tailored interventions. Finally, AI can transform proteomic data into intuitive visual representations. This aids researchers and veterinarians in comprehending complex data patterns and facilitates effective communication of findings.

Development of standardized protocols and data sharing

The field of proteomics has greatly benefited from the development of standardized protocols for sample preparation, data acquisition, and data analysis (Hughes et al. 2019). Standardized protocols have improved the reproducibility and comparability of proteomics experiments, which is essential for the validation of findings and the advancement of the field (Gawor and Bulska 2023). For instance, an extensive database about bovine milk proteins was created and continually updated (Das et al. 2022; Maity et al. 2020a).

The sharing of proteomics data has become increasingly important for the advancement of the field, especially in domestic animals (Almeida et al. 2021). The integration of proteomics data from multiple studies and laboratories can provide a more comprehensive understanding of protein

expression and function in domestic animals (Almeida et al. 2021; de Almeida et al. 2018). This has led to the development of data repositories, such as PRIDE (PRoteomics IDEntifications) and PeptideAtlas, which provide a platform for the sharing and dissemination of proteomics data (Vizcaino et al. 2010). Furthermore, standardized metadata formats such as mzTab have facilitated the integration and analysis of proteomics data from multiple sources (Griss et al. 2014). The ProteomeXchange consortium (www.proteomexchange.org) has developed a standardized metadata format, called mzTab, which provides a uniform way to report proteomics data.

Proteomics experiments generate large amounts of data, which require proper management and analysis. However, ensuring data quality and standardization can be challenging due to several factors. Proteomics experiments are prone to technical variability, such as variations in sample preparation, instrument performance, and data processing (Lippolis and Nally 2018). These variations can lead to differences in the quality and quantity of data generated, making it difficult to compare results across studies or laboratories. To address this issue, it is important to establish quality control measures and standard operating procedures (SOPs) for all aspects of the experimental workflow (Plews et al. 2011). Furthermore, proteomics data are often complex and require sophisticated bioinformatics tools for analysis (Gligorijevic et al. 2018).

However, the lack of standardization in data formats and analysis pipelines can hinder data integration and comparison. Moreover, the limited availability of comprehensive data in public databases for the majority of domestic animal species and their pathogens poses a challenge to the confidence and efficacy of protein identification efforts (Steen and Pandey 2002). To overcome this challenge, efforts are underway to develop standardized data formats and analysis workflows, such as the Proteomics Standards Initiative (PSI) (Taylor et al. 2006). The goal of this initiative is to establish standardized data protocols in proteomics that promote seamless data comparison, exchange, and verification among researchers. Similar efforts should be applied in the veterinary field.

Factors shaping proteomic patterns: challenges and limitations in local breeds

Sample preparation and variability

Sample preparation and variability pose significant challenges in proteomics research in domestic animals, mainly in local breeds (Lippolis and Nally 2018). Sample preparation involves multiple steps, such as collection, storage, extraction, purification, fractionation, and labelling of proteins or peptides from biological samples (Bodzon-Kulakowska et al. 2007; Lippolis et al. 2002). Each step introduces variability due to differences in protocols, equipment, reagents, environmental conditions, or human errors (Bandow 2010). Moreover, biological samples from domestic animals can be complex and diverse due to factors like species, breed, age, sex, health status, diet, or environmental exposure, which can impact protein expression and modification profiles.

Species and breed

Different species and breeds of domestic animals can have distinct genetic backgrounds, physiological characteristics, and metabolic pathways, which can result in differences in protein expression and modification profiles. For instance, a study on chicken breast muscle proteomics and genomic showed significant differences in gene expression and protein abundance between three different chicken breeds (Zhang et al. 2019).

Age and sex

Age and sex can also influence the proteomic profiles of domestic animals. For example, sex-specific proteins, such as arginine esterase, which is similar to human prostate-specific antigen, comprise a significant portion of total urine proteins in adult intact males, in contrast to females and castrated males (Isani et al. 2018). A study aimed to assess meat quality traits and the expression of specific genes involved in the tenderization process in the *longissimus lumborum* (LL) muscle of young male goats at different ages. Results showed differences in carcass characteristics, meat quality traits, and gene expression among different age groups (Sacca et al. 2019).

Tissue and cell types

The diversity of tissues and cell types within an organism adds another level of complexity. Different tissues and cell types have distinct protein expression profiles and may require different sample preparation methods. For example, the protein composition of blood plasma is very different from that of muscle tissue. Therefore, it is essential to carefully select the appropriate sample type and prepare it accordingly for the specific research question.

Health status

The health status of domestic animals, such as their immune status or disease condition, can also impact proteomic

profiles. For example, proteomic analysis of saliva demonstrated significant changes in protein expression levels in response to different infections, indicating the influence of health status on proteomic profiles (Lamy and Mau 2012; Thomas et al. 2009).

Stage of disease

The clinical stage of a disease can also has a significant impact on the proteomic profiles of domestic animals. For instance, a study on urinary proteomics in cattle infected with a bovine spongiform encephalopathy (BSE) showed that stage of the disease influenced the urinary proteome, leading to the identification of a specific panel of proteins that exhibited high accuracy in discriminating between control and infected cattle (Plews et al. 2011).

Diet and environmental exposure

Diet and environmental exposure can affect the proteomic profiles of domestic animals through changes in nutrient intake, toxin exposure, or environmental stressors. A proteomics approach was used to study the effects of peripubertal feeding on heifer mammary development (Daniels et al. 2006). Mammary tissue extracts from Holstein heifers under different dietary treatments and body weights were analysed using 2-dimensional protein maps. Changes in protein expression were observed in response to dietary treatment and body weight. Mass spectrometry was used to identify the proteins, such as transferrin, which plays a role in insulin-like growth factor binding protein-3 binding. This study highlights the significant impact of nutrition on the protein profiles of the mammary gland in heifers.

It is important to consider these factors when designing proteomic studies in domestic animals to account for potential variability in protein expression and modification patterns. Proper sample selection, study design, and statistical analysis can help mitigate the impact of these factors on proteomic results.

Conclusion

In conclusion, proteomics is amenable to ethical guidelines for the use of animals in research in a variety of different environments and with an emphasis on animal and veterinary research in the tropics and subtropics. These often include 3Rs principle, reduction, refinement, and replacement. Thus, we can gain a deeper understanding of physiological and pathological processes in domestic animals. Exciting trends like multi-omics integration, single-cell proteomics, and proteogenomics hold great promise improve animal health, welfare, and productivity, of particular relevance to tackle specificities of tropical diseases and parasitism. As such, collaborations, standardizing protocols and sharing data become possible. In addition, developing animal-specific resources, such as databases and analytical tools, will ultimately overcome limitations such as resources availability. This review integrates information that is important for our understanding of the proteomic molecular mechanisms underpinning animal health and disease, and therefore has implications for therapies.

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Declarations

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