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Proteomic studies in pregnant and lactating cows. A review

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ABSTRACT. Proteomics, an innovative branch of science, deals with comprehensive analysis of protein expression at a particular time in a given biological system. Proteomics enables analysis of the repertoire of proteins, comparison of protein profiles, and reveals changes in expression. Proteomic studies may be useful in solving scientific problems and may have applications in practice. The use of proteomic techniques is increasing in relation to farm animals in order to assess their health status, growth rate and productivity, but it is worth emphasizing that still is inadequate to needs. The detection of biomarkers enables early diagnosis, prevention, and finding therapeutic solutions. During puberty in heifers and during the first pregnancy and lactation, dynamic and gradual adaptive changes in the intensity of metabolic processes and changes in the activity of regulatory mechanisms are observed. These changes are associated with the growth and development of the foetus, preparation of the mammary glands for lactation, and, after parturition, with reproductive system regeneration and preparation for new fertilization and pregnancy. Comparison of protein profiles and identification of differentially expressed proteins involved in particular metabolic pathways may be useful in comprehensive analysis of functional changes in pregnant and lactating cows. Current knowledge, results of scientific studies, and their application into practice indicate that proteomics will introduce new standards into physiological research in the near future. This paper presents studies on pregnant and lactating cows, with emphasis on proteomics of blood serum/plasma, foetal membranes, liver, amniotic fluid, allantoic fluid, uterine fluid, urine, mammary gland, milk, and comparative proteomics of healthy and sick cows.

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Introduction

During puberty, pregnancy and lactation, dynamic and gradual adaptive changes occur in the female body. An organism adapts through changes in the intensity of metabolic processes and the activity of its regulatory mechanisms. These changes are connected with the growth and development of the foetus, preparation of the mammary gland for lactation, and, after parturition, with reproductive system regeneration and preparation for new fertilization and pregnancy.

Analysis of protein profiles and identification of proteins involved in particular metabolic pathways may be useful in broadening the knowledge about changes in an organism during this period. Proteomics makes it possible to analyse the repertoire of proteins, compare protein profiles and reveal changes

in expression. Identification of proteins, that differ in their expression, and analysis of their function, permits selection of biomarkers and helps to better understand the functional changes in pregnant and lactating cows.

Proteomic tools might be applicable in clinical studies searching for differences in the expression of proteins (with emphasis on biomarker discovery) from tissues and biological fluids of healthy and unhealthy organisms. These analysis enable development of diagnostic methods revealing pathological changes before any clinical symptoms occur (Shankar et al., 2005; Thadikkaran et al., 2005; Herosimczyk et al., 2006).

Proteomic techniques are still rarely applied in physiological studies in farm animals, even though analysis of protein repertoires is unparalleled and might be useful, not only in broadening knowledge, but also might be applicable in animal husbandry-veterinary practice (Talamo et al., 2003; Nair et al., 2004; Lippolis and Reinhardt, 2008; Skrzypczak et al., 2011; Herosimczyk et al., 2013).

There are few reports on the application of proteomics in pregnant and lactating cows. Most studies have been performed on women and laboratory animals. Proteomic techniques were used, for instance, in analysis and diagnosis of disturbances in pregnant women and their foetuses. In humans, biomarkers have now been identified for: pre-eclampsia (Blumenstein et al., 2009; Gharesi-Fard et al., 2010), preterm parturition (Horgan et al., 2009), intra-amniotic infection (Gravett et al., 2004), ectopic pregnancy (Gerton et al., 2004), preterm rupture of membranes (Michel et al., 2006) and foetal growth restriction (Gupta et al., 2006).

Proteomic techniques have been used in analysis of changes in the function and regression of the corpus luteum during the end of pregnancy and onset of lactation in rats (González-Fernández et al., 2008), stress-induced changes in the hippocampus of pregnant rats (Ardekani et al., 2011), effect of a high-protein diet on feed intake and liver metabolism during pregnancy and lactation and after weaning in mice (Kuhla et al., 2010). The mammary gland in women, mice and rats and milk from women, sows, goats, mares, monkeys, ewes, mice has been investigated using proteomics (Roncada et al., 2002; Fortunato et al., 2003; Jacobs et al., 2004; Miranda et al., 2004; D'Auria et al., 2005; Davies et al., 2006; Mengé et al., 2008; Boumahrou et al., 2009; D'Alessandro et al., 2010; Jin et al., 2010; Moreira et al., 2010; Wu et al., 2010). Proteomic studies have also focused on pregnancy-related proteins from the pig uterus endometrium (Chae et al., 2011), uterine luminal fluid from early pregnant ewes (Koch et al., 2010) and the serum repertoire of proteins during the periparturient period in sheep (Chiaradia et al., 2012).

Analysis of the literature shows that in pregnant and lactating cows (healthy or with disturbances), proteomic tools have been applied in studies regarding identification of proteins and changes in their expression in blood serum/plasma, amniotic fluid, allantoic fluid, urine, foetal membranes, uterine tissues, and liver.

Proteomics of blood serum/plasma

Jin et al. (2005) was one of the first to apply proteomic techniques in analysis of blood serum protein profiles from Holstein cows during pregnancy. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was used for identification. The main purpose of this analysis was to compare the proteomes of 9 pregnant and 8 non-pregnant cows and to identify proteins characteristic for early pregnancy. Blood serum proteins were separated with the use of two-dimensional electrophoresis (2DE). Between days 21 and 31 of pregnancy, 9 proteins (including transferrin, albumins, IgG, gamma globulins) showed different expression. These authors stressed that despite these proteins being abundant serum proteins, their molecular weights and pI values differ from those of the main serum proteins. An important achievement of this study is its presentation of the profiles of key proteins involved in early pregnancy, which might be useful in detection of early pregnancy.

Cairoli et al. (2006) analysed blood serum profiles from 9 Holstein heifers during pregnancy and post partum. The authors applied one-dimensional electrophoresis (1DE), 2DE, and tandem electrospray high-performance liquid chromatography-mass spectrometry (HPLC-MS). Comparison of proteomes revealed that the most abrupt changes in protein expression occur at the end of pregnancy and early post partum. Increases in the expression of kininogen were observed until the fifth month of pregnancy, as well as abrupt changes of alpha-2-HS-glycoprotein and apolipoprotein A-IV that decreased at the end of pregnancy and early post partum. A reverse tendency was observed for alpha-1-antichymotrypsin. Expression of orosomucoid and haptoglobin significantly increased during the last days of pregnancy and after parturition. Analysis of these proteins may help in identification of animals at risk for post partum infections.

Comprehensive studies of changes in the expression of plasma proteins in heifers before insemina-

tion, during pregnancy, and in the first months of lactation were conducted by Jarosz (2013) and Kurpińska (2013). Proteomic techniques enabled development of protein maps and analysis of changes in protein expression in pregnant and lactating cows. Differentially expressed proteins were analysed according to their physiological function. They were involved, for instance, in lipid transport (clusterin precursor, apolipoprotein A-IV precursor, apolipoprotein A-I precursor, apolipoprotein E precursor), immunological response (conglutinin precursor, complement C4 precursor, mannose-binding protein C precursor), blood clotting and acute phase response (fibrinogen alpha chain precursor, fibrinogen gamma B-chain precursor, fibrinogen beta chain precursor, fibrinogen like protein-1 precursor, endopin-1 precursor), tissue remodeling and protection (72 kDa type IV collagenase precursor, actin cytoplasmic 1, Kelch-like ECH associated protein 1, pigment epithelium-derived factor precursor, gluthathione peroxidase 3 precursor). Analysis of changes in expression, together with analysis of biochemical indicators, enabled monitoring changes in metabolic processes during the first pregnancy and months of increasing milk production.

Li (2012) applied 2DE, isobaric tags for relative and absolute quantitation (iTRAQ) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) in blood serum proteome studies in Holstein cows during the periparturient period. The author identified 78 proteins, of which 19 showed changes in expression. For instance, conglutinin, apolipoprotein A-II, deoxyhaemoglobin, and ECM1 showed higher expression, whereas haptoglobin and lipopolysaccharide-binding protein showed lower expression one day after parturition in comparison with day 21 before delivery. It is worth stressing that the results of this analysis may add to the knowledge about immune system function at the end of pregnancy and early post partum.

Yang et al. (2012) conducted proteomic studies on blood plasma from primiparous Holstein cows at the end of pregnancy and during the first few weeks of lactation. Application of 2DE, Western blot, and liquid chromatography coupled with tandem mass spectrometry in healthy cows revealed that 14 protein spots showed higher expression on the day of delivery compared with day 21 before parturition. Expression of orosomucoid, haptoglobin and amyloid A increased until parturition and then decreased. Transthyretin showed the reverse tendency. Apolipoprotein E and immunoglobulin gamma 1 were upregulated post partum, as compared with the prepartum period. Changes in expression of proteins during the periparturient period are significant and should not be considered a result of pathological processes. The studies cited above on blood serum/plasma support this conclusion. Analysis of these changes might give an insight into the physiological changes connected with parturition.

Lippolis and Reinhardt (2005) isolated neutrophils from the blood of healthy cows one month before parturition. Using one-dimensional electrophoresis followed by reverse-phase chromatography in line with electrospray tandem mass spectrometry, the authors identified more than 250 proteins of various functions involved in: cell mobility (e.g., beta-actin, profilin I), immunological response (e.g., calgranulin B, lactotransferrin precursor), signaling (e.g., septin 6), transport (e.g., albumin precursor, annexin A1), DNA and protein synthesis (e.g., histones), and metabolism (e.g., α-enolase, cystatin C precursor). The authors emphasized that periparturient immunosupression impaired neutrophil functions. These studies broaden the knowledge about transient immunosuppression in cows.

Proteomics of the placenta and liver

Kim et al. (2010) using 2DE, MALDI-TOF MS and LC-MS/MS demonstrated changes in protein expression in the placenta of healthy cows. Among 2000 resolved protein spots, 273 proteins were identified. A reference protein map of the cow placenta was created. The results (including changes in expression of proteins like leucine aminopeptidase, JM-27 protein, cytochrome P450, MGC157400 protein), add to the knowledge about placenta function in both normal and complicated pregnancies.

Other studies on the placenta proteome were also performed by Kim et al. (2005). Placentas were obtained from cows after somatic cell nuclear transfer (SCNT), and from cows after artificial insemination. Application of 2DE, MALDI-TOF MS, and Western blotting revealed that 60 protein spots showed different expression between the groups. In the first group, 33 protein spots were upregulated (e.g., interleukin-18, annexin-XI, tissue inhibitor of metalloproteinase 2), and 27 protein spots were downregulated (e.g., vimentin, tropomyosin beta chain). The identified proteins were grouped according to their function: proteins involved in repair and protection of proteins, signal transduction, transcription regulation, differentiation of proteins, ion transport, immunological response, and metabolism. There were also proteins that were components of the cytoskeleton and extracellular matrix. These results revealed the composite profiles of key proteins involved in the abnormal placenta derived

from SCNT, and suggested abnormalities in expression of these genes in the SCNT placenta, resulting in foetal losses after SCNT.

Klisch et al. (2006) observed changes in glycosylation in pregnancy-associated glycoproteins and prolactin-related protein I, synthesized in binuclear trophoblast giant cells. Western-blot and immunoenzymatic tests showed that before delivery in cows, the molecular mass of these proteins is reduced (1–2 kDa) in comparison with late pregnancy. These changes, connected with modification of asparagine-linked glycans, can modify the properties of the proteins. These analysis might be valuable for diagnosis of pregnancy in cattle.

Kuhla et al. (2009) conducted proteomic studies on liver from Holstein first-lactation cows. They used 2DE, Western blot, and MALDI-TOF MS to analyse the molecular mechanisms of metabolic adaptation and control of energy homeostasis. Two groups were created, cows fed *ad libitum* and feed-deprived. Thirty-four proteins showed different expression between the groups. Feed deprivation caused downregulation of proteins associated with fatty acid oxidation, glycolysis, electron transfer, protein degradation, and antigen processing, as well as cytoskeletal rearrangement. Upregulation was observed for enzymes of the urea cycle, fatty acid or cholesterol transport proteins, an inhibitor of glycolysis, and previously unknown changes in the calcium signaling network. The results indicate that feed deprivation causes lower metabolic activity of the liver. These results might be useful in broadening knowledge about hepatic lipidosis and maintaining energy homeostasis.

Proteomics of amniotic, allantoic and uterine fluids, and urine

Riding et al. (2008) analysed the proteome of amniotic and allantoic fluids from cows in the first trimester of pregnancy (about 45 days of pregnancy). Proteomic analysis was performed with the use of 2DE, matrix-assisted laser desorption ionization-time of flight tandem mass spectrometry (MALDI-TOF-MS/MS), liquid chromatography electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS), and liquid chromatography-matrix-assisted laser desorption ionization-time of flight-tandem mass spectrometry (LC-MALDI-TOF-MS/MS). The authors demonstrated differences among the proteomes of these fluids (the proteome of amniotic fluid was least complex). Most proteins from both fluids were involved in transport, metabolism, and development of the embryo. For example, in allantoic fluid cathepsin I, cystatin B and C, alpha 1-antitrypisin, tissue inhibitor of metalloproteinase 2, ubiquitin B precursor, proteins connected with foetus protection, and inhibitors of serine and cysteine proteases were detected. Higher expression of osteonectin and procollagen type I alpha 1 and type III alpha 1 in amniotic fluid in comparison with allantoic fluid was observed. These results are useful in deeper understanding of the role of amniotic and allantoic fluids in the proper growth and development of the foetus.

Morris et al. (2007) using 2DE and liquid chromatography-mass spectrometry (LC/MS) with linear ion trap, analysed the effect of stage of cycle on the bovine uterine fluid proteome. The authors confirmed the high dynamics of changes depending on the phase of the cycle and intrauterine environment. For example, aldose reductase, plakoglobin, heat shock protein 27 (HSP-27) showed higher expression on day 15 of the cycle in comparison with day 3. These studies give an insight into intrauterine changes depending on the day of the cycle and intrauterine factors, which might be useful in analysis of problems with fertility, conception and implantation.

Munoz et al. (2012) conducted proteomic studies (two-dimensional difference gel electrophoresis (2D-DIGE), Nano-LC-ESI/MS/MS) on uterine fluid from pregnant cows in order to analyse the interaction between mother and embryo at the early stage of pregnancy. Fifty protein spots showed different expression between pregnant and non-pregnant cows. Proteins such as glycolysed tumor necrosis factor alpha (TNFα), interleukin 1 beta (Il-1B), insulin, lactotransferrin, non-phosphorylated peroxiredoxin, albumin, purine nucleoside phosphorylase, HSPA5 and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) showed lower expression in pregnant cows. Higher expression was observed for phosphorylated-peroxiredoxin, annexin A4 and nonglycosylated-TNFα. The authors emphasize that embryos enhanced the embryotrophic ability of uterine fluid and decreased uterine protein, but blood progesterone was unaltered. The embryonic signaling agents involved may be TNFα and IL-1B, either alone or in cooperation with other proteins.

Minhas and Saxena (2008) analysed uterine fluid from infertile cows (sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and native electrophoresis). The authors detected protein of a mass lower than 10 kDa, which was not present in healthy non-pregnant and pregnant cows. That protein was considered a potential marker of repeat breeding. Other results showed that two proteins having molecular weights of 27 and 24 kDa

were only present in uterine fluid from healthy, non-pregnant cows. Proteins with molecular weights of 20, 22, 33, 52, 68 and >100 kDa were never found in pregnant cows. These studies provide additional information about infertility in cows.

Proteomic studies (2DE, Western blot, MALDI-TOF MS/MS) of urine from pregnant cows allowed Pyo et al. (2003) to identify and characterize bovine pregnancy-associated protein. The authors determined its molecular weight (21 kDa) and isoelectric point (6.1). Higher expression of this protein was observed in urine of pregnant cows, but its urinary concentration was not correlated with the duration of the pregnancy.

Proteomics of the mammary gland and milk

Proteomic techniques (2DE, mass spectrometry) have also been applied in the analysis of changes in mammary tissues during lactation, thus confirming that these techniques enabled broadening the knowledge about bovine mammary gland development. Daniels et al. (2006) claimed that the protein composition of mammary gland parenchyma depended on physiological condition and nutrition. Their studies were carried out on heifers fed two different diets; the nutrients intake supporting 650 or 950 g of daily gain. Samples were also collected at body weights of 200 and 350 kg. Dietary treatment influenced 131 protein spots and 108 protein spots were altered by body weight. Changes in expression were found in: fascin, protein disulphide-isomerase, aldehyde dehydrogenase 1, 26S proteasome regulatory subunit, proteasome subunit β type-2, glutathione S-transferase pi, selenium-binding protein.

Beddek et al. (2008) analysed mammary gland tissues in cows between day 120 and 151 of lactation in order to detect enzymes involved in metabolic pathways for synthesis of milk molecules, including fatty acids and lactose. With the aid of 2DE and MALDI-TOF MS the authors detected 11 of the 15 enzymes in the direct pathways for conversion of glucose to fatty acids, 2 of the pentose phosphate pathway enzymes (uridinediphosphogalactose-4-epimerase, UDP-glucose pyrophosphorylase), and 2 of the enzymes for lactose synthesis from glucose (transketolase, 6-phosphogluconate dehydrogenase).

Peng et al. (2008) using SDS-PAGE and reversed-phase liquid chromatography/electrospray ionization tandem mass spectrometry (RPLC-ESI-MS/MS), analysed microsomes from mammary tissue to discover proteins that affect lipid metabolism. The authors detected 703 proteins, including 160 predicted transmembrane proteins. The proteins were grouped according to their subcellular localizations and biological functions. More than 50 proteins were associated with cellular uptake, metabolism and secretion of lipids. The authors suggest that the results might be useful in better understanding of gene expression and tissue remodeling in lactation.

Yang et al. (2009a) analysed the membrane fraction of the mammary gland of Holstein cows in order to explain the molecular mechanisms of host defense response in the protection of the mammary gland. Using SDS-PAGE and an ion trap mass spectrometer equipped with HPLC, the authors identified 183 proteins and classified them according to their physicochemical properties. Molecular weights ranged between 4.87 kDa (casocidin-I) and 325.29 kDa (alpha 3 type VI collagen isoform 2 precursor) and isoelectric points between 4.27 and 11.75 (ribosomal protein L13). The identified proteins were involved in different processes, e.g., binding of ions, proteins, DNA and lipids; adhesion of cells, signaling, and signal transduction. It is worth emphasizing that these data may provide valuable information for broadening the knowledge about the mechanisms of mammary gland milk secretion and infection, and may enable identification of potential protein targets for therapies.

Rawson et al. (2012) with the aid of 2D-DIGE compared enzyme abundances between the liver and mammary gland of lactating Friesian cows. The authors determined quantitative differences in protein amounts. In the liver, the abundance of enzymes catalyzing gluconeogenesis and β-oxidation was higher, and in mammary gland tissue, the abundances of enzymes were consistent with fat synthesis rather than β-oxidation.

Han et al. (2008b) applied 2DE, MALDI-TOF MS and Western blot to identify early pregnancy-specific milk whey proteins by comparing samples from Holstein cows during pregnancy (days 30 and 50) and from non-pregnant cows. Twelve spots were differentially expressed among 500–600 resolved. These proteins were identified as lactoferrin, NADH dehydrogenase subunit 2, albumin, serum albumin precursor and transferrin. These studies might be useful for early diagnosis of pregnancy.

Other studies performed by Han et al. (2008a) on milk from pregnant (early pregnancy, day 30 and 50) and non-pregnant cows, revealed that among 600–700 protein spots resolved in gels, changes in expression were observed in 16. The authors suggest that proteins specific to pregnancy are serum albumin precursor, IgG1 heavy chain constant region, conglutinine precursor, epithelial keratin10 and kelch-like ECH-associated protein. Molecular masses and isoelectric points of those

proteins differed from abundant milk or serum proteins, which suggests that these proteins may be pregnancy-specific subunits or fragments of albumin and IgG.

Changes in the whey proteome (of colostrums and mature milk) from healthy Holstein-Friesian cows in early lactation were observed by Senda et al. (2011). The main changes in expression were noted during the first 48 h after parturition. Many proteins were more abundant in colostrum than in milk. The authors identified 25 proteins from 100 protein spots resolved in gels. Proteins such as zinc-α-2-glycoprotein, vitamin D-binding protein, immunoglobulin G2 chain C and β2-microglobulin were observed only in colostrum. It was emphasized that most of the minor whey proteins in colostrum are very important to the passive immunity of newborn calves, and some of them take part in nutritional supplementation of the neonate.

Comparative proteomics in healthy and unhealthy cows

Xia et al. (2012) analysed the blood plasma proteome from healthy Holstein cows and ones affected with milk fever. By using 2D-DIGE and MALDI-TOF MS the authors identified 8 out of 23 protein spots with variable expression. They represented 5 different proteins. The upregulated proteins were identified as angiotensin and endopin 2B. The downregulated ones were serum albumin, fibrinogen beta chain and IgG heavy-chain C-region.

Choe et al. (2010) with the aid of 2DE, western-blot and MALDI-TOF MS, performed proteomic studies on endometrium sampled from healthy cows and cows with endometritis. In the endometritis group, higher expression was noticed for desmin, α-actin-2, HSP-27, peroxiredoxin-6, luteinizing hormone receptor isoform 1, collectin-43 precursor, deoxyribonuclease-I, and major histocompatibility complex (MHC) class I heavy chain. Lower expression in this group was exhibited by transferrin, interleukin-2 precursor, haemoglobin β subunit, and potassium channel tetramerisation domain-containing 11. The authors claim that desmin and α-actin-2 may play important endometritis-related roles, and could be useful markers for the diagnosis of bovine endometritis and, additionally, they suggest that desmin, α-actin, HSP27, HSP70 and MHC-I may be important in preparing the endometrium for implantation.

Xu and Wang (2008) using 2DE and MALDI-TOF-TOF compared liver proteomes from healthy and ketotic cows (28 days after parturition). Thirty-eight proteins were differentially expressed in these groups. They were involved in energy metabolism, carbohydrate degradation, fatty acid metabolism, amino acid metabolism, antioxidation, cell structure, nucleotide metabolism and protein metabolism (e.g., arginase 1, creatine kinase M-type, elongation factor-Tu, flavin reductase, mioglobin).

Yang et al. (2009b) with the aid of 2DE and an ion trap mass spectrometer equipped with an HPLC system compared mammary tissues from healthy cows and clinically mastitic cows. Six proteins were differentially expressed in both groups, 5 of them were identified (in the first group: haemoglobin beta, kappa-casein and tryptophanyl-tRNA-synthetase; in the second group: haemoglobin beta, cytochrome C oxidase and annexin V). These proteins are involved in binding, transport and catalytic activity. These results may broaden the knowledge about the host mammary immune system response to defense against pathogens at the protein level and give additional information about potential protein targets for treatment. Other studies by Yang et al. (2009c) demonstrated higher expression of haptoglobin precursor and lower expression of secretoglobin, family 2A, member 1 in blood plasma from mastitic cows in comparison with healthy cows.

Wang et al. (2010) performed proteomic studies (2DE, ion trap mass spectrometry equipped with a surveyor HPLC system) of nuclei of mammary tissue from healthy cows and cows with clinical mastitis. The differentially expressed proteins were involved in the formation of the cellular skeleton, metabolic regulation, and apoptosis regulation. In mastitic cows, 7 proteins were upregulated (e.g., collagen type I alpha 1, ceratin, HSP, glyceraldehyde 3-phosphate dehydrogenase) and 2 were downregulated.

According to Smolenski et al. (2007) proteomic analysis of milk may be a valuable source of information about the condition of the mammary gland. The authors compared milk proteomes of healthy Friesian cows and clinically mastitic ones (on the first day after parturition and week 10 of lactation). With the aid of 2DE, MALDI-TOF MS, and LC-MS/MS they identified 95 proteins. At least 15 of them were involved in host defense (e.g., lactoferrin, lactoperoxidase, HSPs, endoplasmin precursor, immunoglobulins). It was concluded that the proteome of milk is very complex and that a significant fraction of minor milk proteins is involved in protection against infection.

The milk proteome was also analysed by Danielsen et al. (2010). The authors infused lipopolysaccharide (LPS) to induce udder inflammation in Holstein-Friesian cows. The samples were collected 3 h before the infusion and 4 and 7 h after it. Changes in expression were observed for 49 proteins

involved in the acute phase response, immunoglobulins, proteins associated with blood clotting, immunosuppression, caseins, chemokines, ion chelates, neutrophil derived peptides and anti-inflammatory proteins. For example, apolipoproteins and C4 and C3 complement factors were upregulated.

Application of proteomics in the future

The number of genes encoding proteins is significantly lower than the number of proteins, therefore, studies carried out only at the genomic level are inadequate to fully characterize the diversity of the 'protein products' of its expression. Analysis of the dynamically changing proteome allows for better understanding of the function of both healthy organisms and changes in it during disturbances.

Proteomic studies might be useful in solving scientific problems, as well as may be applicable in veterinary medicine practice. Application of proteomic techniques is increasing in farm animals in order to asses health status, growth rate and productivity. It is essential to compare proteomes between healthy and diseased individuals, as this helps identify biomarkers. Proteomics aims at early diagnosis, prevention, and finding therapeutic solutions with selective drugs and gives the possibility of tracing and shaping signaling pathways, as well as forecasting and monitoring therapy response.

It must be stressed that good design of experiments, proper selection of proteomic techniques and statistical methods, as well as in-depth knowledge about the limitations of these techniques are crucial if valid results of studies are to be obtained. A limitation in studies on cattle is the still poorly characterized *Bos taurus* genome. Another challenge is reproducibility and resolution of mixtures of proteins and peptides, especially these of low molecular weight and low abundance. Standardized collection of biological material and accuracy in sample manipulations are vital for elimination of artifacts in analysis. Intra- and inter-variability between samples must also be taken into account.

Ongoing improvement of proteomic techniques, current knowledge, results of scientific studies and their application into practice indicate that proteomics will introduce new standards into physiological research in the near future.

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