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Bioactive components of mare colostrum whey

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Abstract. Colostrum is particularly rich in proteins and bioactive peptides that play an important role in the health of newborns. Proteomics can help identify and characterise these components, which is crucial for understanding their effects on newborns. The study material consisted of colostrum collected 12 h after delivery from six primiparous Polish noble half-bred mares. Two-dimensional electrophoresis and mass spectrometry were used to separate, identify and quantify mare colostrum whey proteins and peptides. The analysis revealed that the largest group comprised extracellular proteins (49% of the total), followed by cytoplasmic proteins (31%). The biological processes in which the identified proteins were primarily involved included cellular component organisation, response to stress, establishment of localisation, defence response, plasma

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membrane-bound cell projection organisation, cell projection organisation, regulation of protein metabolic process, response to biotic stimulus. The proteins were primarily associated with the following molecular functions: small molecule binding, ion binding, molecular function regulator activity, cation binding, enzyme regulator activity, carbo-hydrate derivative binding, enzyme binding, signalling receptor binding, calcium ion binding, lipid binding, cytoskeletal protein binding, molecular function activator activity. Knowledge of the protein composition of mare colostrum whey is crucial for understanding the role of colostrum in the healthy development of newborns. Due to differences in colostrum protein composition between individual horse breeds, further proteomic research is needed to characterise these differences and assess their implications.
Key words: bioactive peptides, mass spectrometry, two-dimensional electrophoresis

Introduction

Mare colostrum is the first milk produced after birth that is a rich natural source of valuable nutrients and biologically active components. Proteins are among its major constituents and include immunologically and biologically active factors (Sheng and Fang, 2009).

Due to the epitheliochorial placenta, foals are born with trace amounts of antibodies. Colostrum is rich in antibodies, particularly immunoglobulin G (IgGs), which are essential for the foal's passive immunity and protection against infections until its own immune system develops (Jeffcott, 1975). Trypsin inhibitors present in colostrum prevent the digestion of immunoglobulins by blocking trypsin activity, allowing antibodies to remain intact (Trzebiatowski et al. 2022). However, the ability of the foal's intestinal mucosa to absorb immunoglobulins declines rapidly and is largely lost by approximately 24 h after birth.

Milk proteins can be divided into three main fractions: caseins, whey proteins and milk fat globule membrane proteins. Mare's milk, unlike cow's milk, is characterised by a higher proportion of whey proteins and is classified as albumin-type milk, similar to human milk (Blanco-Doval et al., 2024b). Whey proteins play an important role in the growth and development processes of suckling mammals.

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The main whey proteins in mare's milk are α-lactalbumin (LALBA) and β-lactoglobulin (LGB) (Potočnik et al., 2011). The average content of LALBA in mare's milk is 29% of the total whey protein content, compared to 54% in cow's milk (Potočnik et al., 2011). LALBA is involved in lactose biosynthesis, which provides the primary source of energy for the foal, as well as plays a role in ion transport, particularly calcium (Permyakov, 2020). Mare's milk contains 31% LGB in the whey protein fraction, which is slightly higher than in cow's milk (20%) but more than twofold lower compared to goat's and sheep's milk (Potočnik et al., 2011). This protein has antiviral properties, stimulates the secretion of pro-inflammatory cytokines, and acts as a transporter of retinol and fatty acids (Tai et al., 2016).

Moreover, mare's milk also contains proteins with antibacterial, antiviral and antioxidant properties, including lactoferrin and lysozyme, which account for approximately 8% and 7% of the total whey protein content, respectively (Markiewicz-Keszycka et al., 2013).

Other bioactive components present in mare's milk include epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1), which play an important role in the growth and development of newborns (Claeys et al., 2014).

In many species, whey proteins contain numerous low-abundance proteins that can be identified using proteomic techniques (Chen et al., 2023). Most of these proteins are involved in biological processes related to immune regulation and metabolic activity (Sun et al., 2023; Castillo-Lopez et al., 2025).

We hypothesised that bioactive peptides associated with the healthy development of newborn foals would be present in mare's colostrum, including those involved in the immune response, organ and system maturation, and milk synthesis and secretion. The identification and characterisation of

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the bioactive compounds of mare colostrum whey may help clarify their role in foal nutrition and disease prevention and may support the identification of biomarkers of animal health. Therefore, this study aimed to determine the protein composition of colostrum collected 12 h after delivery from primiparous Polish Noble Half-bred mares.

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Material and Methods

Animals, Study Design and Sample Collection

The experiment was performed on six primiparous mares of the Polish Noble Half-Blood breed from the horse stud in Nowielice. The mares were kept in a stable-pasture system and had constant access to feed and similar levels of physical activity. Colostrum was collected 12 h after delivery in early spring, from March to April. The initial colostrum fractions (approximately 50 ml per teat) were collected and subsequently pooled. Immediately after collection, the colostrum samples were cleaned of environmental contaminants (hair, skin) and stored at –80 °C until proteomic analysis.

After thawing, the samples were defatted by removing the lipid layer formed on the surface after centrifugation for 30 minutes at 4 °C at 4500 rpm. Caseins were then precipitated by gradually adding 30% acetic acid to each sample until a pH of 4.6 was reached. The samples were subsequently centrifuged (4 °C, 3380 rpm, 15 min), and the supernatant was precipitated with chilled acetone (−20 °C). The resulting protein precipitate was dissolved in a lysis buffer containing 7 M urea, 2 M thiourea, 4% w/v CHAPS, 1% w/v DTT, 40 mM Tris, and 2% w/v Biolyte. Before proteomic analyses, protein concentration in the samples was measured using the Protein-Assay (Bio-Rad, Hercules, CA, USA).

Two-Dimensional Electrophoresis (2-DE)

For isoelectric focusing (IEF), 800 µg of protein was mixed with a lysis buffer to a final volume of 650 µl and applied to 4–7, 24 cm ReadyStrip™ IPG Strips (Bio-Rad, Hercules, CA, USA). IEF was performed using Protean® IEF Cell (Bio-Rad, Hercules, CA, USA) by ramping the voltage to a maximum of 5000 V until a total of 90,000 Vh was reached, with a maximum current of 50 µA per strip. After IEF, the IPG strips were reduced with DTT (1% w/v) for 15 min and then alkylated with iodoacetamide (2.5% w/v) for an additional 20 min, both in the equilibration buffer (6 M urea, 0.5 M Tris/HCl, pH 6.8, 2% w/v SDS, 30% w/v glycerol). After the equilibration step, the IPG strips were transferred onto 12% polyacrylamide gels (20 × 25 cm) and the two-dimensional electrophoresis was performed at 40 V for 3.5 h, and subsequently at 100 V for 17 h at 15 °C using a Protean Plus™ Dodeca Cell™ electrophoretic chamber (Bio-Rad, Hercules, CA, USA). Finally, Coomassie staining of gels was carried out as described previously by Pink et al. (2010).

Mass Spectrometry and Bioinformatic Data Analysis

Protein spots were manually cut out from the polyacrylamide gels and decolourised by washing in buffer containing 25 mM NH_4HCO_3 in 5% (v/v) acetonitrile (ACN), followed by two washes with a solution of 25 mM NH_4HCO_3 in 50% ACN. The gel pieces were dehydrated (100% ACN), vacuum dried and incubated overnight with trypsin (20 µl/spot containing 12.5 µg trypsin/ml in 25 mM NH_4HCO_3; Promega, Madison, USA) at 37 °C.

Two different matrix-assisted laser desorption/ionisation–time of flight mass spectrometry instruments were used to identify protein spots: the Microflex™ MALDI-TOF mass spectrometer

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(Bruker Daltonics, Bremen, Germany) and the 7T solariX™ MALDI FT-ICR mass spectrometer (SolariX 2xR, Bruker Daltonics, Bremen, Germany).

The Microflex™ MALDI-TOF was operated in a positive-ion reflector mode. External calibration was performed using Peptide Mass Standard II (Bruker Daltonics) based on monoisotopic values of seven peptides: angiotensin II (1046.5420 Da), angiotensin I (1296.6853 Da), substance P (1347.7361 Da), bombesin (1619.8230 Da), ACTH clip 1-17 (2093.0868 Da), ACTH clip 18-39 (2465.1990 Da) and somatostatin 28 (3147.4714 Da), whereas internal calibration was based on the tryptic autolytic products (842.51 and 2211.10 m/z). The mass spectra were acquired with 150 shots of a nitrogen laser operating at 20 Hz, as previously described by Dratwa-Chałupnik et al. (2020).

Spots that were not successfully identified with the Microflex™ MALDI-TOF mass spectrometer were further analysed using the 7T SolariX 2xR MALDI FT-ICR mass spectrometer equipped with a dual ESI-MALDI source and a smartbeam™ II 1 kHz, 355 nm solid state Nd:YAG laser focused to a diameter of ~25 μm. The mass spectra were collected in a positive-ion mode with 1000 laser shots per spot. Internal calibration was performed using a known m/z value, and external calibration was carried out using sodium formate (NaFA) clusters. Data were acquired with the use of ftControl and analysed using the Data Analysis software (Bruker Daltonics, Bremen, Germany).

Peptide mass fingerprinting (PMF) data were compared with vertebrate databases (SWISS-PROT; http://us.expasy.org/uniprot/ and NCBI; http://www.ncbi.nlm.nih.gov/) using the MASCOT search engine (http://www.matrixscience.com/) in Protein Scape 4.2 software (Bruker Daltonics Bremen, Germany). Search criteria included trypsin as the enzyme, carbamidomethylation as a fixed modification, and oxidation (M) as a variable modification for the Microflex™ MALDI-TOF MS

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data; deamidation (NQ), oxidation (M) and Gln→pyro-Glu (N- term Q) were set as variable modifications for the SolariX MALDI FT-ICR MS data. Peptide mass tolerance was 50–150 ppm, with a maximum of two missed cleavage sites. Contaminating peaks of keratin and trypsin were removed manually from the peptide mass list prior to database search. The results of PMF-based identification were accepted when the protein score was significant ($P < 0.05$) with at least five matching peptides and 15% sequence coverage.

Gene ontology (GO) analysis was performed for specific gene products of *Equus caballus* using the online bioinformatic software DAVID (https://david.ncifcrf.gov). The identified gene products were categorised according to their involvement in biological processes and metabolic functions. A network of interactions between identified mare colostrum proteins was constructed using the STRING v.12 database (https://string-db.org/), and the cellular location of the proteins was determined using the UniProt database (http://www.uniprot.org/).

Results

Two-Dimensional (2-D) Gel-Based Profile of Mare Colostrum Proteins

We used 2-DE coupled with MALDI-TOF and MALDI FT-ICR mass spectrometry to establish a 2-D map of proteins expressed in mare colostrum whey. A total of 489 protein spots were matched across the gels of colostrum samples collected 12 h after calving (mean CV = 83.55%). Protein spots with similar staining intensity and location on each gel (240) were selected for the mass spectrometry analysis. In total, 115 spots were successfully identified, which corresponded to 52 different gene products. A representative gel image of proteins present in mare colostrum whey is shown in Figure 1, with all identified spots marked by numbered circles. Most of these spots (73%) were identified as equine-specific (*E. caballus*, *E. asinus*, *E. przewalski*) based on a Mascot score

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greater than 59, sequence coverage of 11–93%, and 5–89 matched peptides. A comprehensive list of all identified proteins, the number of matched peptides, sequence coverage, Mascot scores, experimental and theoretical pI values and molecular weights, along with the taxonomy is given in Supplementary Table S1. The highest proportion of proteins had a molecular weight of 50–150 kDa, followed by proteins with a molecular weight ranging from 20 to 37 kDa.

The 2-D gel analysis showed that 16 proteins were present as multiple spots, indicating that they were different proteoforms, whereas the remaining 35 proteins were detected as single spots. The most abundant proteins were serum albumin, expressed in 24 spots (spots 12, 14–16, 34–41, 43, 50, 53, 59, 65–71, and 76); LGB-1, detected in 12 spots (17-21,24-28, and 112-113); serotransferrin, detected in 10 spots (1, 42, 44-49, 51, and 56); α-S2 – casein, detected in 5 spots (103-104, 61, 78, and 97); and LGB-2, present in 4 spots (86-89). LALBA (32-33, 96) and liver carboxylesterase (6-8) were each represented by three spots. Two spots each were identified for LALBA A (114, 115), LALBA B/C (23, 31), β-actin (81, 93), β-casein (60, 62), DnaJ homolog subfamily A member (9, 72), immunoglobulin mu heavy chain constant chain secreted form (2, 79), TGF-beta receptor type-1 (74, 77), and vitamin D-binding protein (5, 92).

For each identified protein spot, the theoretical pI and MW values from UniProt or NCBI were compared with the experimental values using the PDQuest 8.01 advanced pI/Mw tool (Supplementary Table S1). Of the 115 identified colostrum protein spots, 37 were classified as significant pI outliers, 25 spots shifted toward the acidic pI range and 12 toward the opposite direction (Figure 2). Experimental MW values differed significantly from the theoretical values for 103 spots; 89 had higher and 14 lower experimental MW values (Figure 3). The pI values ranged from 4.60 (LGB-2, spot 88) to 6.80 (annexin A9, spot 53), while estimated molecular masses ranged

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from 11.90 (LALBA A, spot 114) to 284.10 kDa (DnaJ homolog subfamily A member, spot 9). It should be noted that most of the identified proteins (94%) were high-molecular-weight proteins (> 30 kDa).

Functional GO Analysis of Mare Colostrum Proteins

All identified colostrum proteins were categorised according to their subcellular location, biological processes and molecular function based on UniProtKB database (accessed 25 August 2025; Supplementary Table S2). Extracellular proteins accounted for 49% of the identified proteins, followed by cytoplasmic proteins (31%) (Figure 4). Among extracellular proteins, serum albumin, serotransferrin, LGB, α-lactalbumin, α-S2-casein and immunoglobulins were most abundant, while actin was the main cytoplasmic protein (Supplementary Table S1). among cytoplasmic proteins, cytoskeletal proteins (actin) predominated, Supplementary Table S1 (Link to the supplement: https://mostwiedzy.pl/pl/open-research-data/protein-profile-of-mare-colostrum-whey,729121410710806-0?_share=f2219e839969f8dc).

Using DAVID software (https:// https://david.ncifcrf.gov), the proteins were classified based on their involvement in biological processes (Figure 5) and molecular functions (Figure 6). The main biological processes included cellular component organisation (n = 15, *P*-value = 4.47×10^{-2}), response to stress (n = 11, *P*-value = 2.34×10^{-2}), establishment of localisation (n = 11, *P*-value = 9.47×10^{-2}), defence response (n = 8, *P*-value = 3.01×10^{-3}), plasma membrane bounded cell projection organisation (n = 7, *P*-value = 9.87×10^{-3}), cell projection organisation (n = 7, *P*-value = 1.09×10^{-2}), regulation of protein metabolic process (n = 7, *P*-value = 1.76×10^{-2}), and

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response to biotic stimulus (n = 6, *P*-value = 4.84×10^{-2}). The main molecular functions were small molecule binding (n = 22, *P*-value = 3.90×10^{-3}), ion binding (n = 18, *P*-value = 5.98×10^{-2}), molecular function regulator activity (n = 15, *P*-value = 8.02×10^{-5}), cation binding (n = 15, *P*-value = 8.87×10^{-2}), enzyme regulator activity (n = 9, *P*-value = 9.32×10^{-3}), carbohydrate derivative binding (n = 9, *P*-value = 8.78×10^{-2}), enzyme binding (n = 8, *P*-value = 6.89×10^{-2}), signalling receptor binding (n = 7, *P*-value = 5.73×10^{-2}), calcium ion binding (n = 6, *P*-value = 1.28×10^{-2}), lipid binding (n = 6, *P*-value = 1.69×10^{-2}), cytoskeletal protein binding (n = 6, *P*-value = 4.34×10^{-2}), molecular function activator activity (n = 6, *P*-value = 6.10×10^{-2}).

STRING Analysis of Protein Networks

The protein–protein interactions (PPI) network analysis using the STRING v12 tool (Figure 7) showed statistically significant PPI enrichment among the top 47 proteins for *E. caballus* (PPI enrichment *P*-value < 1.0×10^{-16}). The PPI network was constructed using proteins as nodes and their known and predicted interactions as edges. The network contained 56 observed interactions, compared with 9 expected interactions based on the structural, genomic and biological context of the proteins, indicating that the identified proteins were functionally connected. The PPI analysis showed enrichment for secretory proteins (n = 12, *P*-value = 1.28×10^{-8}), molecular function regulator activity (n = 14, *P*-value = $2{,}74 \times 10^{-2}$), enzyme inhibitory activity (n = 7, *P*-value = $2{,}74 \times 10^{-2}$), and homeostasis (n = 9, *P*-value = $2{,}27 \times 10^{-2}$).

Discussion

Colostrum intake provides essential factors for the health and survival of foals. During the first few h of a foal's life, some colostrum components can be absorbed in relatively intact form,

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providing nutrients needed for growth and development and passive immunity against pathogens until its own immune system matures.

In this study, we aimed to determine the protein composition of colostrum from primiparous Polish Noble Half-bred mares collected 12 h after delivery. Few studies have described the protein composition of equine milk, partly because mare's milk is not commonly used in the dairy industry (Miranda et al., 2004; Barello et al., 2008; Blanco-Doval et al., 2024a). Nevertheless, interest in equine milk is growing due to its lower allergenic properties in humans compared to ruminant milk (cow, sheep, goat). Cunsolo et al. (2017) reported that donkey milk proteins, especially casein fractions, show greater homology to human milk proteins than to bovine proteins.

Among the identified protein spots were LGB and LALBA. These proteins account for approximately 60% of the total protein of horse milk whey. LGB is a globular protein found in the milk of various mammals, except humans, camels, lagomorphs and rodents (Wodas et al., 2020). It is involved in the transport of small molecules and may facilitate the absorption of lipophilic vitamins and affect enzyme activity (Wodas et al., 2020).

Majority of the identified proteins were extracellular, with plasma albumin being the most abundant. A well-known function of albumin is to maintain normal blood oncotic pressure, which is important for regulating the distribution of water between the extracellular and intracellular compartments (Evans, 2002). It also binds and transports various molecules, including metabolites such as cholesterol, fatty acids, cations and anions, gases such as NO, and exogenous substances like drugs and diet-derived compounds (Fanali et al., 2012). Albumin also shows antioxidant, anti-inflammatory, immunomodulatory, and anticoagulant properties (Garcia-Martinez et al., 2015). The role of plasma albumin in milk is not entirely understood. This protein and other blood-derived

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proteins may enter colostrum because the blood–milk barrier is temporarily more permeable during early lactation. Since albumin can bind a variety of ligands, it may also act as a carrier of fatty acids, calcium, and hormones in milk.

Transferrin is another extracellular protein identified in mare's colostrum whey. It is a blood plasma protein responsible for binding and transporting iron ions to tissues (Charan Giri, 2022). Only a small proportion of transferrin in mouse milk is derived from the blood via transcytosis (Zhang et al., 2000), whereas most is synthesised and secreted directly by mammary gland epithelial cells (Lee et al., 1987).

Mare's colostrum contains numerous bioactive peptides that are involved in various metabolic processes. Some of these components may exert their effects after reaching the circulation in an intact or biologically active form. The increased permeability of the intestinal barrier during the first days of life may allow bioactive colostrum components to enter the circulation, potentially contributing to the newborn's development and health.

The α_1-antitrypsin protein found in mare's colostrum is a protease (trypsin) inhibitor produced by the mammary gland (Chowanadisai and Lönnerdal, 2002). It binds tightly to trypsin, limiting its activity in the newborn's digestive tract. This mechanism could protect other bioactive colostrum proteins from proteolytic degradation and allow them to remain biologically active after ingestion (Chowanadisai and Lönnerdal, 2002). In the early neonatal period, the slow maturation of proteolytic enzyme synthesis and secretion in the neonatal gastrointestinal (GI) tract, together with the high concentration of α_1-antitrypsin in colostrum, protect bioactive components of colostrum from digestion. The presence of significant amounts of intact α_1-antitrypsin in the stool of healthy

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breastfed infants indicates limited proteolytic activity in the early stages of development (Davidson and Lönnerdal, 1990).

The present study is the first to demonstrate the presence of B-type natriuretic peptides (BNP) in mare's colostrum. These biologically active peptides belong to the family of natriuretic peptides, which also includes atrial natriuretic peptide (ANP; found in human milk) and C-type natriuretic peptide (CNP) (Ken-Dror et al., 1997; Lemaitre et al., 2022). In the absence of evidence for natriuretic peptide synthesis in the mammary gland, these peptides are thought to be transported from the maternal circulation to the mammary gland. Further research should clarify whether BNP in the mammary gland is transported by blood or produced locally.

ANP and BNP primarily bind to the natriuretic peptide receptor (NPR)-A receptor, initiating a signalling cascade that increases intracellular cyclic guanosine monophosphate (cGMP) levels and results in similar physiological effects, while CNP interacts with the NPR-B receptor, which is also part of the guanylate cyclase system (Lemaitre et al., 2022). Studies have suggested that milk bioactive peptides may regulate the development of mammary glands and other tissues in newborns (Schanbacher et al., 1997). The goat mammary gland expresses NPR-B receptor, indicating that it may be a target organ for the natriuretic peptide system (Fan et al., 2022). Moreover, CNP regulates the expression and secretion of the antimicrobial peptide S100A7 in mammary epithelial cells (MECs) via the NPR-B/JNK/c-Jun signalling pathway, which plays a role in mammary gland innate immunity (Fan et al., 2022).

Physiological functions of natriuretic peptides, particularly their role in milk and infant development, have not been fully elucidated and require further research. BNP is a hormone that helps regulate blood pressure (Cannone and Burnett, 2021), and its presence in colostrum suggests

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a potential role in newborn foal health and development. It may also regulate water balance and the transition from foetal to postnatal circulation. A study in mice has demonstrated that signalling involving CNP and NPR-B is crucial for GI motility because it regulates muscle function and gastric emptying (Sogawa et al., 2013). In NPR-B-deficient (slw/slw) mice, CNP stimulation failed to induce cGMP production or relaxation of the pylorus and large intestine (Sogawa et al., 2013). These mice showed severe GI dysfunction, including milk retention in the stomach and intestinal distension, often leading to death before weaning.

Studies in rats have demonstrated that ANP is synthesised in the gastric mucosa (Vuolteenaho et al., 1988; Li et al., 2006). According to Vuolteenaho et al. (1988), it may play a role in the regulation of water and electrolyte balance in the GI tract.

Moreover, Li et al. (2006) suggested that this natriuretic peptide may also inhibit GI motility, acting not only as a local autocrine and/or paracrine regulator, but also as an endocrine factor. On this basis, BNP identified in mare's colostrum may contribute to the regulation of water and electrolyte balance and intestinal peristalsis in foals. Further research is necessary to confirm this specific role of BNP and clarify its mechanism of action in the digestive system of foals.

Intersectin-2 (ITSN2) is a protein involved in clathrin-mediated endocytosis (Pucharcos et al., 2000) and the regulation of endosomal recycling pathways (Gubar et al., 2020). Epidermal growth factor receptor substrate 15-like 1 (EPS15L), also identified in mare's colostrum, is associated with clathrin-mediated endocytosis and vesicle formation (Tebar et al., 1996). Both ITSN2 and EPS15L have been implicated in the activation and regulation of signalling pathways (Tebar et al., 1996; Gubar et al., 2020). Another protein involved in vesicular transport identified in mare's colostrum is Eps15 homology domain-containing protein 3 (EHD3), which regulates endocytic transport and

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intracellular membrane trafficking (Bahl et al., 2016) and has also been identified in human milk-derived extracellular vesicles (EVs) (van Herwijnen et al., 2016). Vesicular transport enables the transfer of lipids, proteins and signalling molecules, including components involved in nutrient delivery and immune protection, which are important for newborn development.

Carboxylesterase 5A (CES5A) was identified in mare's colostrum for the first time. It is a secreted enzyme belonging to the carboxyesterase family, found in various mammalian tissues, including the brain, kidneys, lungs, and testis. It has also been detected in urine and male reproductive fluids (Holmes et al., 2008). Equine carboxylesterases (CES1, CES2, CES3, CES5) share significant amino acid similarities to their human counterparts, particularly human hepatic CES1 (Holmes et al., 2008). The latter protein has been identified as a component of cytosolic lipid droplets in hepatocytes, where it is involved in lipid metabolism (Lian et al., 2018). CES5A, a secreted form of carboxylesterase, has two reported functions in male mammals: regulating the production of pheromone precursors in urine, and participating in lipid and cholesterol transport in seminal fluid (Miyazaki et al., 2003; Ecroyd et al., 2006). To our knowledge, this is the first report of CES5A identification in colostrum. Its presence may be associated with lipid and cholesterol transport into colostrum, potentially via milk fat globules.

Proteins involved in immune processes, including immunoglobulins, have been detected in mare's colostrum. Because the epitheliochorial placenta allows only minimal transfer of maternal antibodies to the foetus, foals depend on colostrum for passive immunity. Mare's colostrum also contains antibacterial proteins, including collectin-12, neutrophil gelatinase-associated lipocalin isoform X5, and lysozyme. Together, immunoglobulins and antibacterial proteins contribute to immune defence and gut health in foals by helping protect against pathogens.

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Conclusions

In summary, we established a 2-DE map of the mare colostrum whey proteome and identified 52 gene products, including unique bioactive proteins whose presence in mammalian milk has not previously been reported.

The quantitative and qualitative composition of colostrum proteins is species-specific and depends on the neonate's needs. The protein profile of colostrum may also vary between breeds, supporting further investigation of mare colostrum from different breeds. Comparative analyses could help identify proteins specific to particular species or breeds.

By accounting for species and breed differences, the present results complement existing data and provide a basis for future proteomic research in this field.

Conflicts of interest

The Authors declare no conflict of interest.

Supplementary material

The supplementary material containing data on the bioactive components of mare colostrum whey is available at https://doi.org/10.34808/erdq-c327 and https://mostwiedzy.pl/pl/open-research-data/protein-profile-of-mare-colostrum-whey,729121410710806-0?_share=f2219e839969f8dc

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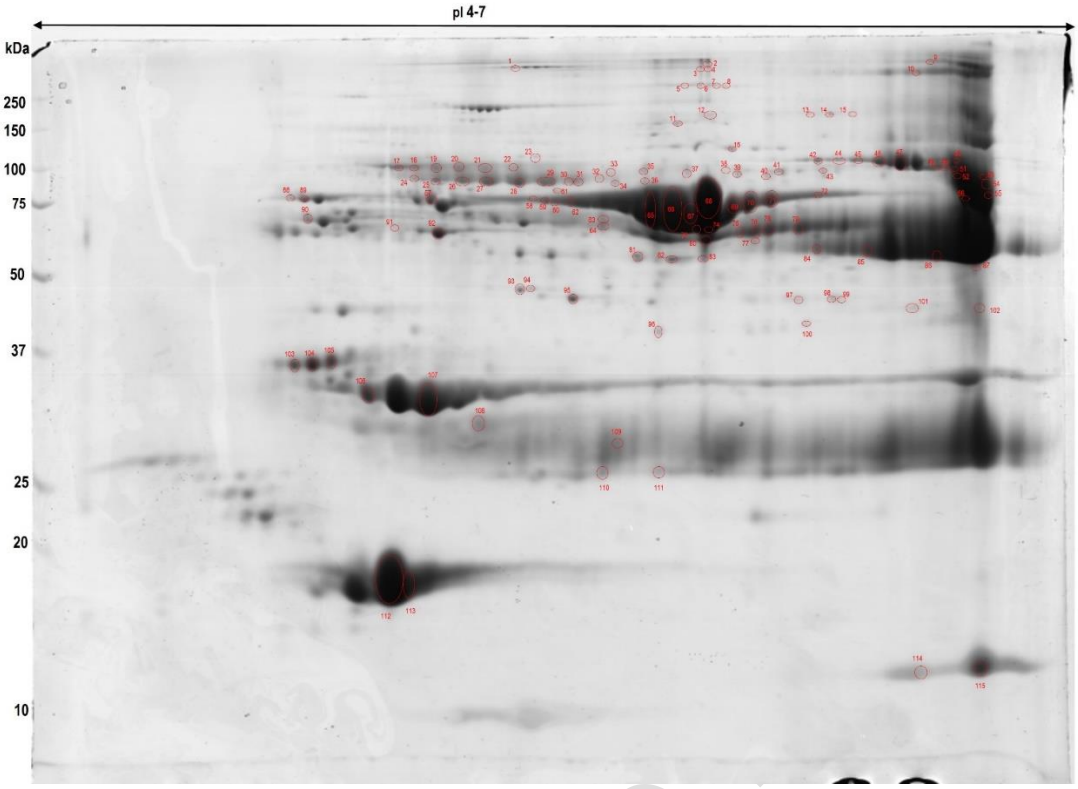


Figure 1. Representative 2-D gel image of mare colostrum whey protein profile collected 12 h after calving. Samples of 800 µg of protein were applied onto the IPG strip (24 cm, pH 4–7) for the first dimension; the second dimension was performed on 12% SDS-PAGE gels that were subsequently stained with Coomassie brilliant blue G-250. Numbered spots were identified using MALDI-TOF and MALDI FT-ICR MS after in-gel digestion of proteins by trypsin. The red circles indicate all identified spots that comprise 2-DE-based protein map of mare colostrum. Spot numbers correspond to those in Supplementary Table S1. Link to the supplement: https://mostwiedzy.pl/pl/open-research-data/protein-profile-of-mare-colostrum-whey,729121410710806-0?_share=f2219e839969f8dc

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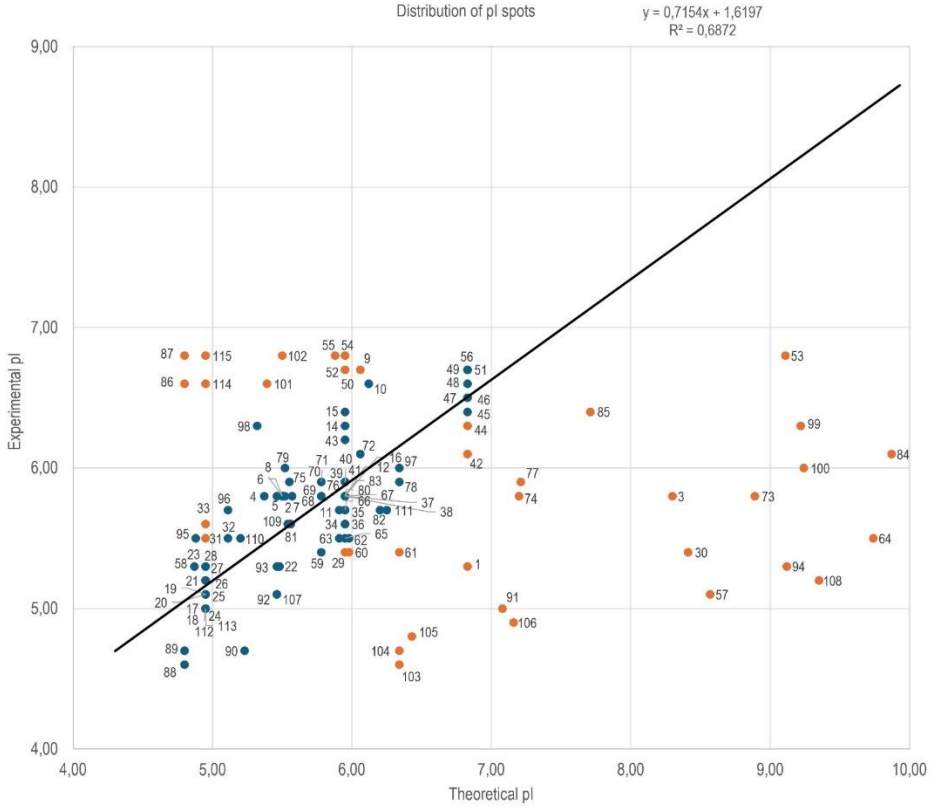


Figure 2. Comparison of experimentally determined and theoretical pI values of the identified protein spots from the mare colostrum whey 2-D protein maps. Spots used for the curve preparation and other plotted spots are marked with blue and orange circles, respectively. Number of protein spots correspond to those in Supplementary Table S1, Figure 1. Link to the supplement: https://mostwiedzy.pl/pl/open-research-data/protein-profile-of-mare-colostrum-whey,729121410710806-0?_share=f2219e839969f8dc

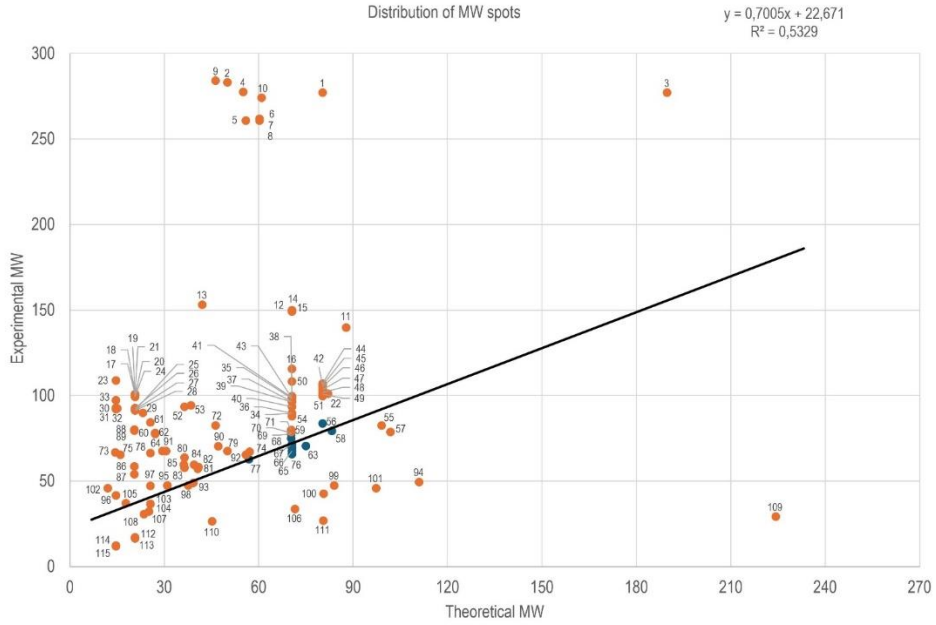


Figure 3. Comparison of experimentally determined and theoretical MW values of the identified protein spots from the mare colostrum whey 2-D protein maps. Spots used for the curve preparation and other plotted spots are marked with blue and orange circles, respectively. Number of protein

spots correspond to those in Supplementary Table S1, Figure 1. Link to the supplement: https://mostwiedzy.pl/pl/open-research-data/protein-profile-of-mare-colostrum-whey,729121410710806-0?_share=f2219e839969f8dc

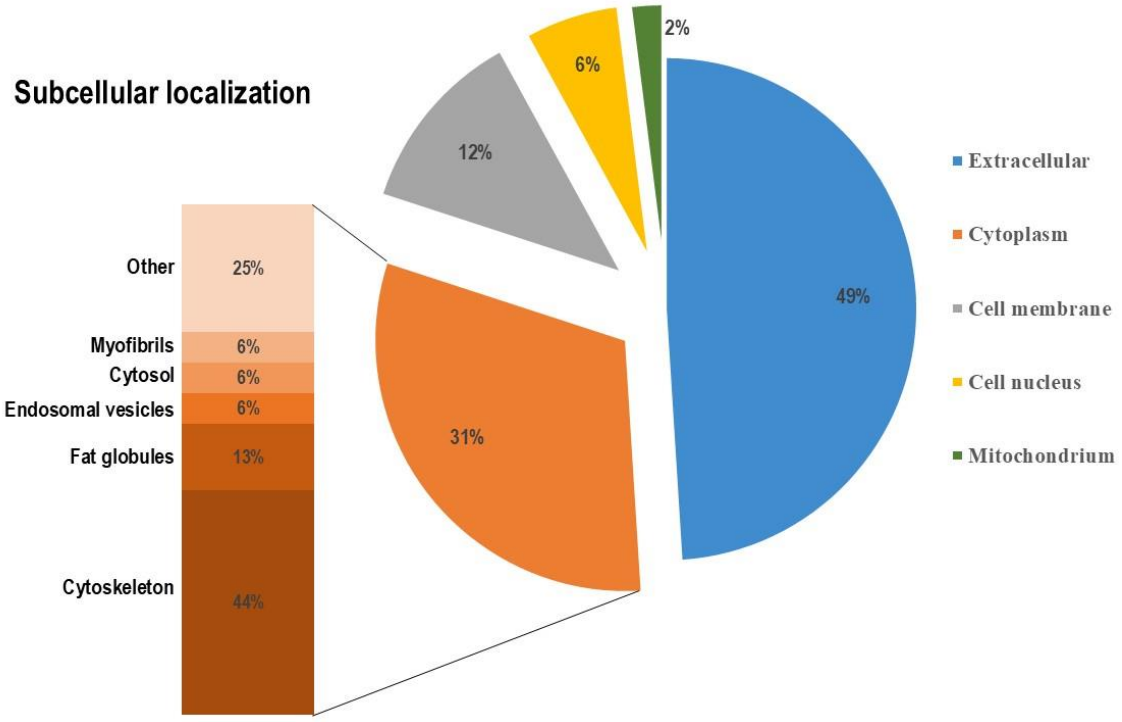


Figure 4. Diagram presenting the percentage of the identified on 2-D maps of mare colostrum whey gene products distribution according to their subcellular localization

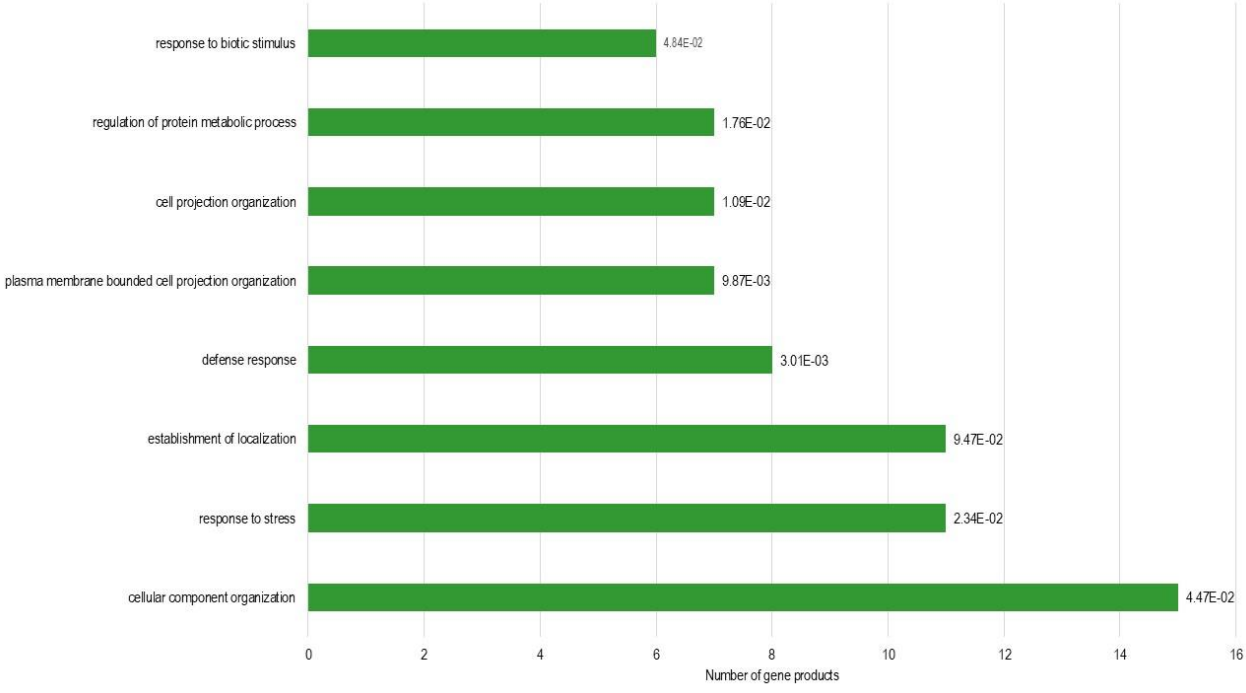


Figure 5. Classification of mare colostrum whey proteins based on Gene Ontology (GO) biological process retrieved from the DAVID database

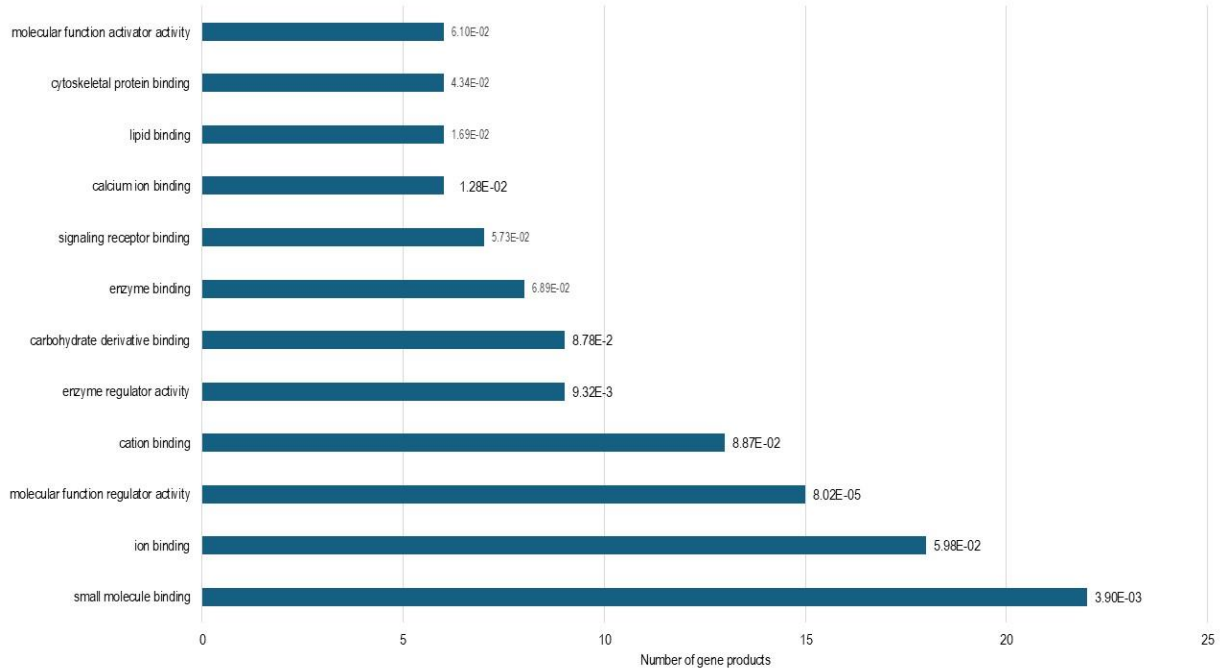


Figure 6. Classification of mare colostrum whey proteins based on Gene Ontology (GO) molecular function retrieved from the DAVID database

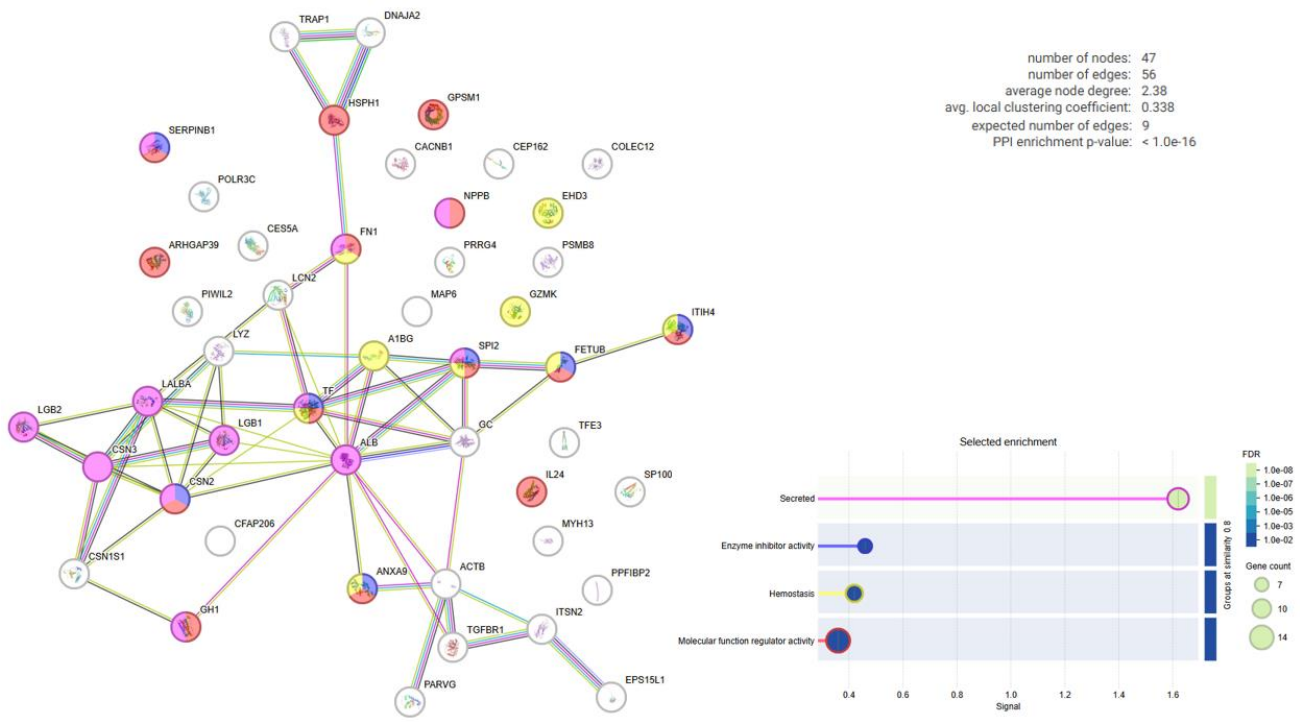


Figure 7. Protein-protein interaction network was constructed using the STRING v12 tool. Each node represents a protein, and each edge represents the interaction between identified proteins found in the mare colostrum. Full name of identified proteins along with the corresponding genes can be found in Supplementary Table S1. Network statistics presenting data concerning included number of nodes and edges, the average node degree, the average local clustering coefficient, the expected number of edges, and the protein–protein interaction (PPI) enrichment *P*-value.

Enrichment analysis dot plot presenting selected terms, protein count in the network represented by the size of each dot, false discovery rate (FDR) indicated through the color coding of the dots. The terms (listed on the Y-axis) are ranked by their enrichment signal of their group. Line color corresponds to the selected term color and is mirrored in the network

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