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Comparative proteomic analysis of low-abundant whey proteins in sow and goat colostrum

Análisis proteómico comparativo de proteínas del suero de baja abundancia en calostro de cerda y cabra

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ABSTRACT

This study compared the whey proteome of sow colostrum (SC) and goat colostrum (GC) using shotgun proteomics combined with protein equalization techniques (CPLL or ProteoMiner™) to enhance the detection of low-abundance proteins. Colostrum samples were pooled from 11 hand-milked sows (20 mL each, collected at 0 h postpartum), while GC samples (2 liters total) were collected from 50 goats during morning milking on day 1 postpartum. A total of 86 low-abundance proteins (<30 kDa) were identified: 26 unique to SC, 32 to GC, and 14 shared. Major whey proteins, including α-S1, α-S2, β-casein, κ-casein, and lactotransferrin (LTF), were present in both colostra. Gene Ontology (GO) enrichment showed that SC proteins were mainly linked to biological regulation (49.04%) and immune response (11.49%), whereas GC proteins were associated with metabolic processes (26.98%) and biological regulation (26.43%), likely reflecting species-specific physiological differences. Protein–protein interaction (PPI) analysis identified highly connected proteins such as LTF, α-lactalbumin, β-casein, Apolipoprotein, and Clusterin in both networks, with additional interactions involving serum albumin in SC. GC displayed unique interactions with Glycam-1, a glycosylated adhesion molecule related to the milk mucin complex, though its immune role remains unclear. These findings provide novel insight into the functional whey proteome of SC and GC, particularly regarding low-abundance proteins, and highlight the value of shotgun proteomics with protein equalization. Surplus goat colostrum emerges as a sustainable heterologous source to enhance piglet survival in hyperprolific systems. The study also underscores the need for parallel gel replicates to minimize technical variability in future proteomic analyses.

Palabras clave:
Ontología génica
Proteoma de la leche
Raza murciano-granadina
Inmunidad pasiva
Cerdos
Interacción proteína-proteína

RESUMEN

Este estudio comparó el proteoma del suero del calostro de cerda (SC) y del calostro de cabra (GC) utilizando proteómica shotgun combinada con técnicas de ecualización de proteínas (CPLL o ProteoMiner™) para mejorar la detección de proteínas de baja abundancia. Se prepararon muestras de calostro agrupadas de 11 cerdas ordeñadas a mano (20 mL cada una, recolectadas a las 0 h posparto), mientras que las muestras de GC (2 litros en total) se recolectaron de 50 cabras durante el ordeño matutino del día 1 posparto. Se identificaron un total de 86 proteínas de baja abundancia (<30 kDa): 26 exclusivas de SC, 32 de GC y 14 compartidas. Las principales proteínas del suero, incluidas α-S1, α-S2, β-caseína, κ-caseína y lactotransferrina (LTF), estuvieron presentes en ambos calostros. El análisis de enriquecimiento de Gene Ontology (GO) mostró que las proteínas de SC se relacionaron principalmente con la regulación biológica (49,04 %) y la respuesta inmune (11,49 %), mientras que las de GC se asociaron con procesos metabólicos (26,98 %) y regulación biológica (26,43 %), lo que probablemente refleja diferencias fisiológicas específicas de cada especie. El análisis de interacción proteína–proteína (PPI) identificó proteínas altamente conectadas como LTF, α-lactalbúmina, β-caseína, apolipoproteína y clusterina en ambas redes, con interacciones adicionales que involucraron albúmina sérica en SC. GC mostró interacciones únicas con Glycam-1, una molécula de adhesión glicosilada relacionada con el complejo de mucinas de la leche, aunque su función inmune sigue sin estar clara. Estos hallazgos proporcionan nueva información sobre el proteoma funcional del suero de SC y GC, particularmente sobre proteínas de baja abundancia, y resaltan el valor de la proteómica shotgun con ecualización de proteínas. El calostro excedente de cabra surge como una fuente heteróloga sostenible para mejorar la supervivencia de lechones en sistemas hiperprolíficos. El estudio también destaca la necesidad de replicados de gel paralelos para minimizar la variabilidad técnica en futuros análisis proteómicos.

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Pig (*Sus scrofa domesticus Erxleben*) and goat (*Capra hircus* L.*)* breeding are fundamental in global livestock production. Pigs are one of the main sources of animal protein; however, their productivity is often constrained by high pre-weaning mortality. This underscores the need for strategies that enhance piglet survival (Martínez-Miró et al. 2020). In turn, goats play a crucial role in dairy production, particularly in Mediterranean regions and developing countries.

Colostrum is a complex biological fluid, rich in components that provide essential functions for neonatal development. Among them, bioactive peptides play a critical role in protecting newborns from pathogens, toxins, and environmental stressors, while also supporting metabolic regulation essential for both physical growth and cognitive development (Ayala et al. 2024).These properties are mainly attributed to milk proteins and peptides that operate through protein—protein interactions. In addition to proteins and peptides, colostrum and milk also contain exosomes and nucleic acids that contribute to neonatal immune development and intercellular communication (Ferreira et al. 2021).

Sow colostrum contains a wide range of whey proteins, including immunoglobulins (IgG, IgA, and IgM), α-lactalbumin, κ-casein, β-lactoglobulin, hormones, growth factors, and enzymes such as lactoperoxidase and lysozyme, in addition to other functional proteins like lactotransferrin. Among these, IgG is the main Ig type (Poonia and Shiva 2022). Goat colostrum produced and stored in the mammary gland during the final 2-3 days of gestation and secreted in the first 2–3 days postpartum is also nutritionally rich. Goat milk proteins are primarily divided into caseins (around 70%) and whey proteins (around 25%); the remaining 5% is made of milk fat globule membrane proteins (Chen et al. 2019).

In pig production, pre-weaning mortality remains a major challenge, largely due to inadequate colostrum availability, especially in hyperprolific sows. These sows, genetically selected for larger litters and leaner carcasses, often cannot produce enough colostrum for their piglets. This results in greater birth weight variability and reduced fat reserves, thus compromising piglet survival (Martínez-Miró et al. 2020). Given their limited energy stores, newborn piglets require a consistent and adequate supply of high-quality colostrum to survive. In contrast, intensive dairy goat systems with mechanical milking and artificial rearing frequently produce more colostrum than needed, thus leading to surpluses that pose economic and environmental disposal challenges. In this context, heterologous passive immune transfer (PIT); that is, the administration of immunoglobulins from one species to another, has gained attention. Studies have demonstrated that ruminant Ig homologs (bovine, caprine, ovine) retain biological activity across species (De Vos et al. 2014; Hernández-Castellano et al. 2016). In pigs, Martínez-Miró et al. (2020) reported successful immune transfer in piglets fed with goat colostrum. Similarly, in a previous study that examined the fatty acid profiles of goat and sow colostrum, it was suggested that goat colostrum could serve as a potential energy supplement for newborn piglets due to its content of short- and medium-chain fatty acids (Ayala et al. 2024). Nevertheless, research on PIT in sows remains limited and further studies are needed to explore its full potential.

Goat colostrum is particularly promising due to its superior digestibility compared to bovine colostrum, and its higher concentrations of free amino acids, magnesium, selenium, and glutathione peroxidase elements that enhance its antioxidant profile (Soloshenko et al. 2020). It also contains medium-chain fatty acids with antibacterial and antiviral properties that are easily absorbed by the neonatal intestine without micelle formation and are a rapid source of energy (Ayala et al. 2024). In addition to immunoglobulins, goat colostrum contains other immune-supporting components, such as lactoferrin and growth factor (Jahan et al. 2017). While immunoglobulins are species-specific, many of these bioactive molecules may not face such immunological barriers (De Vos et al. 2014).

This study aimed to characterize and compare the whey proteomes of sow and goat colostrum using shotgun proteomics combined with protein equalization techniques. Proteomics is a powerful tool to study low-abundance proteins in complex biological samples such as colostrum and milk. Whey protein composition of porcine, bovine, and human milk has been characterized using proteomic approaches (Ogawa et al. 2014;

Chopra et al. 2020; Aslebagh et al. 2023). Although the proteomic composition of porcine colostrum (Ferreira et al. 2021) and caprine colostrum (Akin et al. 2014; Sun et al. 2020) has been independently investigated, a systematic comparative proteomic analysis between the colostrum of these two species is not available in the current scientific literature. To address this gap and explore alternative strategies to enhance piglet survival, this study aimed to characterize and compare the whey proteomes of sow and goat colostrum, investigate their biological functions, and evaluate their protein–protein interaction networks.

MATERIALS AND METHODS

Animals and Management

The study was conducted at two commercial farms in southern Spain. The pig farm was in Huércal-Overa (Almería, Southeastern Spain), with 4000 Large White x Landrace sows, in a one-week batch management system. The sows had free access to water and were fed twice a day. During gestation, they received a commercial diet for pregnant sows (2.5 kg per day) containing 12.2 MJ kg^{-1} metabolizable energy (ME), 130 g kg^{-1} crude protein, and 6 g Lys kg^{-1}. The lactation feed contained 12.9 MJ kg^{-1} ME, 160 kg^{-1} crude protein, and 8 g Lys per kg lysine. The diet composition followed the recommendations of the Spanish Foundation for the Development of Animal Nutrition (FEDNA). In this study, a total of 12 crossbred sows (Large-White Landrace, farrowing range 2 to 6) inseminated with Pietrain sperm were included. During the first 40 days, they were in 2.5-meter-long and 0.63-meter-wide gestation crates; thereafter, in a free-access stall for groups until 110 days of gestation. Sows were then moved to farrowing rooms and housed in individual farrowing crates (2.0x2.5 m) on a slatted plastic floor, providing a minimum space of 2.5 square meters per animal. This complies with European Union regulations for the protection of animals used for experimental and scientific purposes (Directive 2010/63/EU). They stayed in these conditions for the 21 days of lactation. On the day of farrowing, the birthing process was monitored, minimizing any interference with it.

The commercial goat farm was in Mula (Spain) and had a herd distributed in 7 lots of 500 Murciano-Granadina goats with an average production of 513 kg, with a lactation period of 210 days. Goats underwent a dry period of 8-12 weeks prior to calving. They were housed in free-access stalls and received a mixture feed through a unifeed system. The prepartum diet (1.5 kg DM/d; 3.19 Mcal ME/d; 112 g MP/d) and lactation diet (2.5 kg DM/d; 4.41 Mcal ME/d; 205 g MP/d) were formulated according to NRC guidelines (2007). Prepartum diet was supplemented with mono-propylene glycol USP (0.05 mL per animal/d) to prevent ketosis and pregnancy toxemia. During the last month of gestation, an extra-protocol vaccination against *Clostridium* spp., *Salmonella/Chlamydia* spp., *Coxiella burnetii*, and *Staphylococcus aureus* was followed to enhance colostrum immunological quality. Prevention of metritis and mastitis included the administration of cephalosporins that do not cross the blood–milk barrier to avoid antibiotic residues in the colostrum.

Collection and processing of colostrum samples

Eleven samples of hand-milked sow colostrum (20 mL per sow) were obtained immediately after the birth of the first piglet (0h). They were collected from the anterior, middle, and posterior udder and subsequently pooled. Once collected, samples were aliquoted, frozen, and stored at -20 °C until analysis. To maintain proper hygiene, the udder of each sow was washed with warm water and dried before collecting the samples. Two liters of goat colostrum were collected from the tank (4 °C) during the morning milking from 50 goats on d1 postpartum. Samples were transferred to the laboratory on ice, aliquoted into 40-mL bottles, and stored at -20 °C until use. The difference in sampling time reflects species-specific mammary physiology and commercial farm practices. In sows, colostrum is not stored, and milk ejection is brief and pulsatile; each teat functions independently to supply a large litter; thus, sampling must be done within the first 6 hours postpartum to ensure true colostrum collection (Segura et al. 2020). In goats, the mammary gland consists of two large glands with cisterns that store sufficient colostrum for one or two kids. Literature indicates that transition milk begins only after 36 h (Segura et al. 2025). Pooled samples were prepared for a single-replicate proteomic analysis in sow (SC) and goat (GC) colostrum.

Methods used for global protein identification

In this study, whey proteins in sow (SC) and goat (GC) colostrum were compared using "Shotgun proteomics."

It refers to the direct analysis of complex mixtures of proteins to rapidly generate a global profile of the protein complement within the mixture (Ogawa et al. 2014). It was made through multidimensional protein identification technology, which incorporates multidimensional high-pressure liquid chromatography (LC/LC), tandem mass spectrometry (MS/MS), and database search algorithms. To estimate protein assortments, all samples were subjected to SDS-PAGE and LC-MS/MS analysis. Subsequently, due to the challenge of delving deeper into the colostrum proteome and uncovering low-abundance proteins that may be masked by high-abundance proteins, particularly IgGs, a protein equalization treatment method involving combinatorial peptide ligand libraries (CPLL or PM, ProteoMiner™) was employed. This technique is renowned for enhancing the detection of less common proteins (Boschetti and Righetti 2023), and it was used to generate the final list of proteins identified in colostrum samples.

Colostrum protein quantification

The Pierce Coomassie Plus Bradford assay kit (Thermo Fisher Scientific, #23236) was used to quantify the total protein concentration. Following the manufacturer's instructions, a calibration line was prepared with known concentrations of bovine serum albumin. After adding the reagent to both the tubes with the calibration line and the tubes with the colostrum samples, they were shaken, and absorbance readings were taken at 595 nm using the Thermofisher Scientific Genesys 20 visible spectrophotometer. Absorbance values of the samples were extrapolated to the calibration line to determine the concentration of total protein in the colostrum samples. The low-abundance minor proteins were enriched by the ProteoMiner Kit (Catalog#163-3008, BioRad Laboratories, Hercules, CA, USA) per manufacturer's protocols.

Separation of protein fractions

Amicon Ultra 0.5 mL filters (Amicon Ultra 0.5 mL centrifugal filter MWCO 10 kDa) were used to prepare protein–enriched fractions of different sizes from colostrum samples. Following manufacturer guidelines, samples were placed onto the filters and underwent centrifugation at 14,000 g for 20 minutes at room temperature. This process resulted in a filtrate that contained a fraction enriched with proteins having a molecular weight smaller than the filter's cut-off size (10 kDa). Meanwhile, the portion that remained on the filter contained larger proteins. To retrieve this retained portion, the filter was turned upside down into a clean tube and subjected to centrifugation at 1,000 g for 2 minutes.

Protein digestion

Samples were subjected to the standard trypsin digestion procedure with some modifications to identify the proteins present in them. Specifically, about 100 µg of each sample were prepared in 200 µl of 50 mM ammonium bicarbonate buffer pH=8.5, with 0.01% ProteaseMax surfactant (Promega), which enhances trypsin digestion. Samples were subjected to reaction with a reducing agent (20 mM DTT) for 20 min at 56 °C. They were then subjected to a reaction with 100 mM iodoacetamide for 30 min at room temperature and in the dark to block the cysteine residue. Finally, samples were subjected to trypsin digestion using 1 µg of Trypsin Gold Proteomics Grade (approximately a 1:100 w/w ratio with respect to the amount of total protein) for 3 hours at 37 °C. Reactions were stopped by adding formic acid to a final concentration of 0.1% and samples were passed through 0.2 µm filters. Then, they were evaporated to dryness using an Eppendorf Vacuum Concentrator model 5301.

Molecular weight determination by electrophoresis (SDS-PAGE)

One-dimensional analysis (SDS-PAGE) was performed using a denaturing polyacrylamide gel electrophoresis with sodium dodecyl sulphate (SDS), as first described by (Laemmli 1970). Briefly, 18% polyacrylamide Tris-HCl gels (8.3x7.3 cm) were prepared for the Mini-PROTEAN Tetra CellBio-Rad Laboratories (Hercules, CA, USA). Colostrum samples (50 or 100 µg) were mixed with 4 x Laemmli loading sample buffer (Bio-Rad) and then heated at 85 °C for 5 minutes. They were then applied to each lane of the gel and ran until complete separation (approximately 1.5 hours at 150 V) using TGS buffer (25 mM Tris pH=8.3, 192 mM glycine, 0.1% SDS). After completing electrophoresis, gels were washed with MilliQ water 3 times for 5 min under agitation. Gels were then stained with PageBlue protein staining solution (ThermoFisher Scientific, #24620) based on colloidal Coomassie blue G250. Molecular

masses were estimated by running PageRuler Plus Pre-stained protein ladder (ThermoFisher Scientific, 26619). Finally, LabScan software was used to acquire scanned images of the gels, and Image Lab software (Bio-Rad, Alges, Portugal) was used for gel analysis. Results were recorded by capturing images of the assays with an Epson Perfection v39 photo scanner.

Bioinformatics analysis

Bioinformatics tools were used to better understand the differences and similarities in the biological function networks of sow and goat colostrum proteins. Venn diagrams were used to graphically represent the junctions, intersections, and distinctions between colostrum whey proteins using GeneVenn software (https://www.bioinformatics.org/gvenn/). Gene ontology (GO) analyses were enriched using a Database for Annotation, Visualization and Integrated Discovery (DAVID - Bioinformatics Resources (https://david.ncifcrf.gov/) on the levels of biological process, using *sus scrofa* and *capra hircus* genomes as references. Protein–protein interactions (PPI) of expressed whey proteins in colostrum were predicted using Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; version 12.0). STRING networks were calculated with a high confidence score of 0.700 for the entire set of colostrum proteins.

RESULTS AND DISCUSSION

Colostrum is well known for containing a wide range of proteins and bioactive compounds that play key roles in immune response, growth promotion in newborns, and favoring gastrointestinal development and nutrient absorption (Martínez-Miró et al. 2020). In porcine, sow colostrum and milk production are the primary limiting factors that determine piglet survival, growth, and development. Goat milk with high nutritional potential and protein as a key component is an interesting alternative for supplementing neonatal piglets. In this study, sow and goat colostrum were compared using a shotgun proteomics method plus a protein equalization treatment (CPLL or PM, ProteoMiner™) that enhances the detection of low-abundant proteins.

SDS-PAGE analysis

Figure 1 displays the SDS-PAGE whey proteins of SC and GC before and after depletion. The protein profile before treatment with CPLLs (Figure 1A) shows a high intensity of broad bands, particularly in the 15 to 130 kDa area, both in SC and GC. Whey protein profiles after depletion revealed 9 protein bands in SC, with molecular masses between 14 and 80 kDa, and 7 protein bands in GC, with molecular masses between 16 and 86 kDa. These levels were compared among groups (Figure 1B).

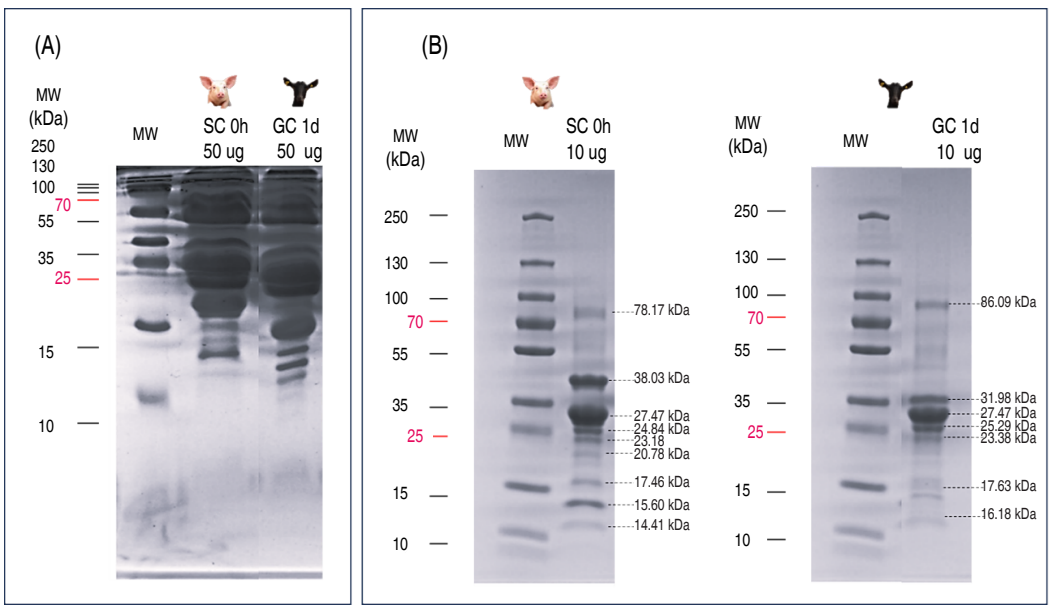

Figure 1. Comparison of SDS-PAGE of colostrum proteins before (A) and after (B) depletion. MW: Molecular weight standards (kDa); SC 0h: sow colostrum samples obtained immediately after the birth of the first piglet; GC 1d: goat colostrum samples obtained on day 1 after parturition.

Protein identification and proteome treatment with CPLL

The protein content of porcine and caprine colostrum postpartum was 116.53 and 75.25 µg µl^{-1}, respectively, measured by the Bradford method (Kielkopf et al. 2020). A total of 143 IDs proteins were preliminarily identified by LC-MS/MS. This number was reduced following CPLL or PM (ProteoMiner) treatment since this study aimed at identifying low-abundance proteins in colostrum. Proteins lacking a gene name or having double names, but the same genes were excluded from the dataset. This list of depleted/enriched proteins was used as input for functional analyses. Figure 2 shows the overlapping Venn diagram of SC and GC whey proteins. Shotgun proteomic analysis after depletion identified a total of 86 less abundant proteins. Out of these, 26 and 32 whey proteins were identified in sow and goat colostrum, respectively, and 14 minor proteins were detected in both (Table 1).

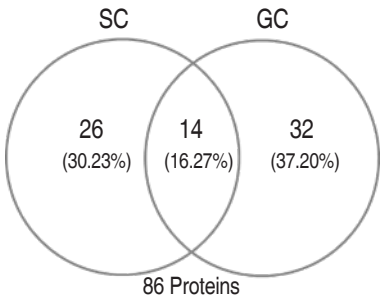

Figure 2. Overlapping Venn diagram of shotgun LC-MS/ MS of minor (<30 kDa) whey protein identifications obtained for sow (SC) and goat (GC) colostrum.

Table 1. List of the 14 common expressed whey proteins identified in porcine and caprine colostrum.

Protein name	Accession number	Score	Molecular weight (kDa)	Coverage (%)	Accession number	Score	Molecular weight (kDa)	Coverage (%)
Proteins identified in both types of colostrum	Sow colostrum 0h [1]				Goat colostrum d1 [2]			
Lactotransferrin	Q6YT39	592.45	77.61	46.7	A3QPC1	969.13	77.33	68.6
Alpha-S2-casein	P39036	439.02	27.57	54.4	P33049	561.18	26.39	91.0
Beta-lactoglobulin-1A/1C	P04119	268.48	19.73	55.0	P02756	270.83	19.98	52.7
Alpha-S1-casein	P39035	251.13	24.15	39.3	A0A0P0EL46	271.97	24.13	79.3
Apolipoprotein A-I	A0A286ZQC7	171.56	25.83	55.2	W5NX51	212.67	29.53	57.9
Ig like domain protein	A0A286ZI90	136.79	67.09	20.9	W5PSQ7	85.68	25.15	19.1
Beta-casein	A0A2C9F376	135.38	25.78	57.5	P33048	287.39	24.86	80.1
Serum amyloid A protein	Q2HXZ9	135.29	14.51	61.5	A5JST2	43.52	14.30	29.6
Peptidoglycan-recognition protein	A0A286ZI97	132.66	24.19	44.1	W5PLB7	133.56	22.63	41,4
Clusterin	K7GND8	108.93	51.90	13.8	W5PZI1	191.17	51.02	22.7
Joining chain of multimeric IgA and IgM	A0A287B5V2	43.94	19.74	18.6	W5PPQ8	96.68	18.14	34.5
Kappa-casein	F1RVB2	30.49	20.97	10.1	L0BU95	215.53	18.01	63.9
Alpha-lactalbumin	P18137	20.84	16.17	19.1	A5JSS8	58.37	16.25	37.3
Polymeric immunoglobulin receptor	A0A287A0N2	10.56	84.70	5.8	W5P9V5	54.07	85.48	6.60

Colostrum proteins from [1] sow and [2] goat colostrum were separated by SDS-PAGE and subsequently gel from CPLL-treated. Peptides masses were searched in the UniProt db. (www.uniprot.org).

Some of the major proteins identified in both sow and goat colostrum included α-S1 casein (24.1 kDa), α-S2 casein (26.9 kDa), β-casein (25.3 kDa), κ-casein (19.5 kDa), and LTF (77.5 kDa). LTF is a glycoprotein and an important host defense molecule that modulates immune function and promotes neuronal development and growth (Jahan et al. 2017). Transferrin is an iron-binding transport protein that can influence the differentiation and proliferation of intestinal epithelial cells, which helps regulate iron absorption and protects newborn piglets against gastrointestinal infections. Due to its non-specific bacteriostatic activity, it is proposed to be used in replacers for newborn piglets. Clusterin, apolipoprotein A1, serum amyloid A protein and peptidoglycan recognition protein were minority functional proteins found in both types of colostrum. They perform functions mainly in the immune response, protein stabilization, and transport regulation (Geraghty et al. 2022). Some of the identified proteins have not been previously described in the colostrum of the species analyzed in this study, or their function in lactation is unknown. These include Matrix Gla protein, Fibromodulin, Papillin, Azurocidin1, among others. It is possible that, as in colostrum and bovine milk, these minority proteins come from the somatic or even epidermal cellular proteome rather than from the proteome expressed in the mammary gland. Unlike other growth factors, such as insulin-like growth factor (IGF), epidermal growth factor (EGF), and non-hormonal bioactive substances (lactoferrin) appear to be synthesized within the mammary gland as well as transported from the maternal circulation (Cunsolo et al. 2015). Differences between SC and GC proteomes stem from variations in metabolic pathways and protein production between monogastric and ruminant animals. Ruminants, through rumen microorganisms, efficiently utilize dietary proteins, non-protein nitrogen, and regenerated urea as nitrogen sources (Hailemariam et al. 2021), resulting in distinct metabolic processes such as sugar fermentation and protein secretion. Additionally, rumen microorganisms convert dietary carbohydrates into short-chain fatty acids (SCFAs) such as acetic, propionic, and butyric acids, leading to lower levels of polyunsaturated fatty acids and glucose compared to monogastric species (Hackmann 2024).

GO Functional Annotation

All identified colostrum whey proteins were characterized using GO functions and classified, according to biological process (BP), into nine groups: (1) biological regulation; (2) immune response; (3) cellular process; (4) biological process; (5) stimulus response; (6) developmental process; (7) localization; (8) metabolic process; and (9) stress response. As shown in Figure 3, GO functional annotations were obtained for the 86 differentially expressed proteins from the comparison between SC and GC. Proteins identified in the SC samples were mainly involved in biological processes related to biological regulation (49%), immune system response (11%), cellular process (10%), and biological processes involved in response to stimulus (7%). Proteins identified in the GC samples were involved in biological processes, including metabolic process (27%), biological regulation (26%), cellular process (24%),

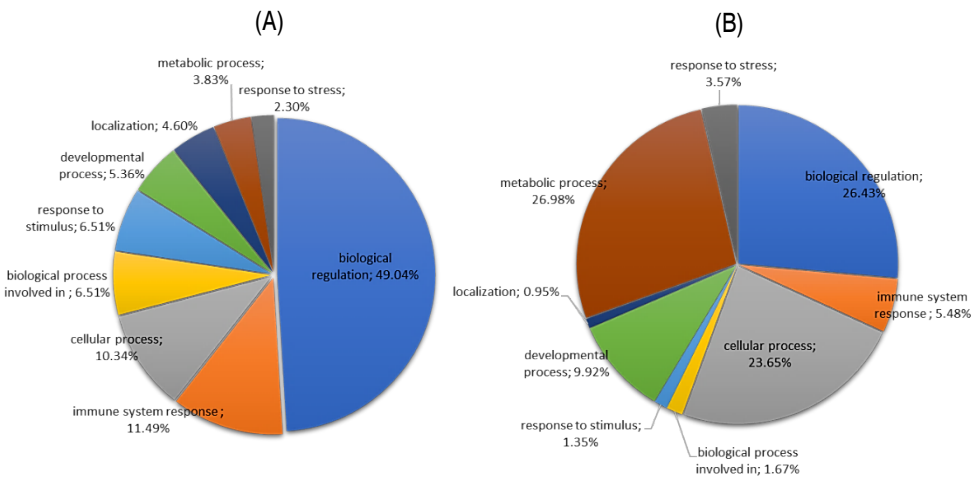

Figure 3. Gene ontology (GO) function annotation of biological process of (A) sow and (B) goat colostrum differential expression proteins.

developmental process (10%), and immune system response (5%). The GO enrichment analysis revealed, until the data set was regularized, that biological regulation and immune response were the most abundant biological processes (BP) of whey proteins in sow colostrum (Figure 3A), accounting for 49.04 and 11.49%, respectively. In goat colostrum (Figure 3B), a large portion of whey proteins were related to biological regulation and cellular processes. In total, these two categories accounted for 26.43 and 23.65%, respectively. Differences in placental structure contribute to this, as goats have a polycotyledonary placenta with distinct areas where the maternal caruncle and fetal cotyledon form placentomes for nutrient and gas exchange. This gives priority to biological and metabolic processes. In contrast, in sows, immunoglobulins do not cross the placenta, and offspring depend solely on colostrum intake at birth for passive immunity transfer (Chavatte-Palmer and Tarrade 2016).

Protein interaction networks (PPI) using STRING program

To better understand the biological potential of SC and GC proteins and growth factors, the protein–protein interaction network database available in STRING software (version 12.0) was retrieved to generate network interaction maps (Figure 4). Protein–protein interaction networks identified a highly connected group of whey proteins in colostrum, including LTF, α-lactalbumin (LALB), and β-casein (CSN2) in both SC and GC (Figures 4A and 4B). LTF, as described above, plays a key immune role. In addition, LALB, a globular protein found in the milk of all mammals, has several bioactivities such as immune modulating, antimicrobial, antiviral, anti-hypertensive, opioid, mineral binding, and antioxidant functions (Kamau et al. 2010). Shared interactions were noted (Tables 2 and 3). The PPI network for sow colostrum (SC) contained 26 nodes connected via 23 edges (Figure 4A). α-lactalbumin (LALBA), Lactotransferrin (LTF), Serum albumin (ALB), and β-casein (CSN2) were the most interacting proteins, with six interactions each, followed by Kappa-casein (CSN3) and β-lactoglobulin- 1A/1C (PAEP) with five interacting proteins. Finally, Alpha-S1-casein (A0A5S6G633_PIG), Apolipoprotein E (APOE), Apolipoprotein A-I (APOA1), and Clusterin (CLUL1) with 4, 3, 2 and 1, respectively (Table 2). Serum albumin (ALBs) plays important roles in lactation and mammary gland health. Although it is not synthesized within the mammary gland, it is proposed to facilitate the transport of long-chain fatty acids and small molecules from the maternal circulation to the neonate via milk. Despite its relatively lower abundance compared with proteins such as α-lactalbumin or lactoferrin, albumin constitutes a functional component of the nutritional profile of milk and of the mammary gland biology. Moreover, its concentration in milk has been observed to increase under conditions such as mastitis or during mammary involution, suggesting a potential involvement in inflammatory responses and tissue repair processes (Rezaei et al. 2016). Casein micelles, colloidal aggregates of proteins and calcium phosphate, maintain a dynamic equilibrium with the aqueous phase of milk, modulating the exchange of components according to physicochemical conditions. Beyond their structural role, they serve as carriers of essential nutrients, primarily calcium, phosphorus, and proteins, essential for neonatal growth and immunonutrition (Broyard and Gaucheron 2015). Clusterin is a multifunctional glycoprotein implicated in epithelial differentiation and morphogenesis. It plays a regulatory role in mammary gland development, with marked expression at the end of pregnancy and again at the onset of involution (Itahana et al. 2007). Regarding whey proteins in goat colostrum (GC), the PPI network contained 26 nodes connected via 16 edges (Figure 4B). Lactotransferrin (LTF) was the most interacting protein with six interactions, followed by α-lactalbumin (LALBA), β-casein (CSN2), and β-lactoglobulin (PAEP) with four interacting proteins. Finally, Clusterin (LOC102182127) and Apolipoprotein A-I (APOA1) with 2 and 1 interactions, respectively (Table 3). The GC network also revealed glycosylated cell adhesion molecule-1 (Glycam-1) interactions with α-lactalbumin (LALBA), β-casein (CSN2), and β-lactoglobulin (PAEP). It is still not clear whether Glycam-1 has an immunological function in milk; however, recent studies (Valk-Weeber et al. 2021) have detected it in both the soluble whey fraction and the milk fat globule membrane (MFGM), indicating multiple roles within the milk matrix. This dual localization suggests its participation in mucin-like complexes in the aqueous phase and its contribution to the structure and function of the MFGM—potentially stabilizing fat globules, mediating interactions with immune receptors, and modulating the availability of glycans to the neonatal gut microbiota. Taken together, these observations point to nutritional and immunological relevance, although their mechanisms of action remain under investigation.

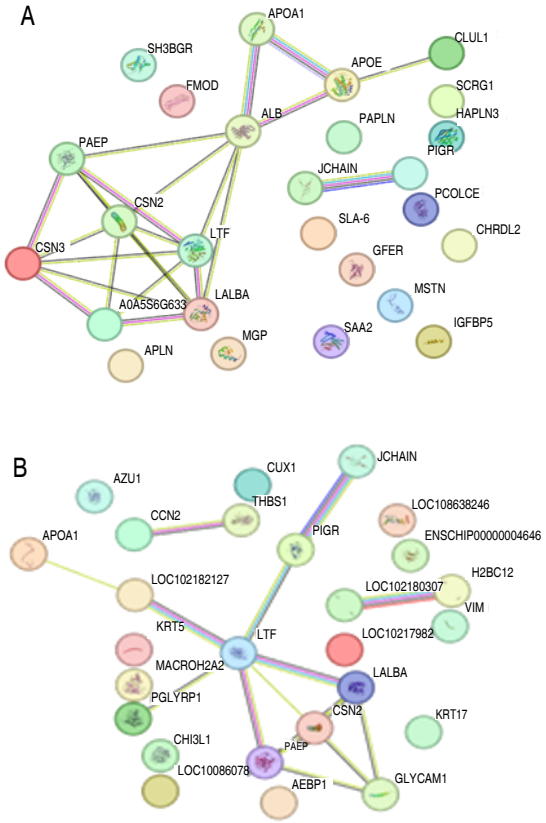

Figure 4. Protein–protein interaction (PPI) network connectivity of the differentially expressed proteins in colostrum; (A) PPI network of whey proteins identified in sow colostrum; (B) PPI network of whey proteins identified in goat colostrum. Interaction score: medium confidence 0.7. Each node represents a protein, the node-node connection represents the interaction between two proteins, and different line colors indicate different interaction types.

Table 2. Analysis of the protein–protein interaction (PPI) network of whey proteins identified in sow colostrum.

ID	Function	Nodes[1]
ENSSSCP00000024183, LALBA	Regulatory subunit of lactose synthase.	ALB, PAEP, CSN2, CSN3, LTF, A0A5S6G633_PIG
ENSSSCP00000026769, LTF	Transferrins are iron-binding transport proteins.	ALB, LALBA, CSN2, CSN3, PAEP, A0A5S6G633_PIG
ENSSSCP00000009875, CSN2	Important role in the determination of the surface properties of the casein micelles.	ALB, LALBA, LTF, PAEP, CSN3, A0A5S6G633_PIG
ENSSSCP00000053712, ALB	The main protein of plasma. It has a good binding capacity with water, Ca (2^{+}), Na ($^{+}$), K ($^{+}$), fatty acids, hormones, bilirubin, and drugs.	LTF, LALBA, CSN2, PAEP, APOA1, APOE
ENSSSCP00000009882, CSN3	Stabilizes micelle formation, thus preventing casein precipitation in milk.	PAEP, LTF, CSN2, LALBA, A0A5S6G633_PIG
ENSSSCP00000044067, PAEP	The primary component of whey. It binds with retinol and is probably involved in the transport of that molecule.	ALB, LTF, LALBA, CSN2, CSN3

Table 2

ID	Function	Nodes[1]
ENSSSCP00000009877, A0A5S6G633_PIG	Function not found.	CSN2, CSN3, LTF, LALBA
ENSSSCP00000003343, APOE	A protein associating with lipid particles, which mainly functions in lipoprotein-mediated lipid transport between organs via the plasma and interstitial fluids.	APOA1, ALB, CLUL1
ENSSSCP00000033805, APOA1	Participates in the reverse transport of cholesterol from tissues to the liver for excretion by promoting cholesterol efflux from tissues and by acting as a cofactor for the lecithin cholesterol acetyl transferase (LCAT).	ALB, APOE
ENSSSCP00000042972, CLUL1	Function not found.	APOE

[1] LALB = Alpha-lactalbumin; PAEP = Beta-lactoglobulin-1A/1C; CSN2 = Beta-casein; CSN3 = Kappa-casein; LTF = Lactotransferrin; ALB = Serum albumin; A0A5S6G633_PIG = Alpha-S1-casein; APOA1 = Apolipoprotein A-I; APOE = Apolipoprotein; CLUL1 = Clusterin. [2] NA, not annotated.

Table 3. Analysis of the protein–protein interaction (PPI) network of whey proteins identified in goat colostrum.

ID	Function	Nodes[1]
ENSCHIP00000016996, LTF	Iron-binding transport proteins.	LALBA, CSN2, PAEP, PGLYRP1, LOC102182127, PIGR
ENSCHIP00000024154, LALBA	Regulatory subunit of lactose synthase.	LTF, CSN2, PAEP, GLYCAM1
ENSCHIP00000024801, CSN2	Important role in the determination of the surface properties of casein micelles.	LTF, LALBA, PAEP, GLYCAM1
ENSCHIP00000012525, PAEP	Primary component of whey. It binds with retinol and is probably involved in the transport of that molecule.	LTF, LALBA, GLYCAM1, CSN2
ENSCHIP00000011297, GLYCAM-1	Function not found.	LALBA, CSN2, PAEP
ENSCHIP00000006353, LOC102182127	Functions as an extracellular chaperone that prevents aggregation of nonnative proteins. Prevents stress-induced aggregation of plasma blood proteins.	APOA1, LTF
ENSCHIP00000010067, PIGR	Function not found.	LTF, JCHAIN
ENSCHIP00000023988, APOA1	Function not found.	LOC102182127
ENSCHIP00000032505, JCHAIN	Function not found.	PIGR
ENSCHIP00000010519, PGLYRP1	Pattern receptor that binds to Murein Peptidoglycans (PGN) of Gram-positive bact.	LTF

[1] LALB = Alpha-lactalbumin; CSN2 = Beta-casein; PAEP = Beta-lactoglobulin; PGLYRP1= Peptidoglycan-recognition protein; LOC102182127 = Clusterin; PIGR = Polymeric immunoglobulin receptor; LTF = Lactotransferrin; GLYCAM-1 = Glycosylation-dependent cell adhesion molecule 1; APOA1 = Uncharacterized protein; JCHAIN = Joining chain of multimeric IgA and IgM. [2] NA, not annotated.

CONCLUSION

This study provides novel insight into the biological relevance of low-abundance colostrum proteins in porcine and caprine colostrum by highlighting their potential biological roles in neonatal development and defense against pathogenic infections. A total of 86 differentially expressed low-abundance proteins (<30 kDa) were identified; 16% were commonly expressed whey proteins shared between both species, including lactotransferrin (LTF), a key immune-modulating glycoprotein. Sow colostrum collected immediately after parturition (0h) had high levels of proteins linked to immune function and neonatal growth, whereas goat colostrum obtained on day 1 postpartum contained numerous proteins involved in metabolic regulation and antibacterial activity, thus contributing to the protection of both neonates and the mammary gland. Notably, certain defense-related proteins, such as β2-microglobulin and Glycam-1, were uniquely detected in goat colostrum, thus suggesting species-specific immune components. From a practical perspective, these insights may support strategies for neonatal nutrition and the design of colostrum-based supplements to enhance early immune protection in livestock. Further investigation, including validation of the immunological functionality of these proteins in animal models or clinical trials, is warranted. Hence, this study confirms the usefulness of shotgun proteomics for characterizing complex colostrum proteomes and presents the first comparative analysis of sow and goat colostrum, thus providing a new perspective on the potential use of heterologous colostrum to address shortages in hyperprolific sows. It also underscores the value of incorporating parallel gel replicates in future work to minimize technical variability and improve the robustness of proteomic analyses.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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