Proteomic analysis of whey proteins in the colostrum and mature milk of Xinong Saanen goats

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ABSTRACT

Xinong Saanen goats are among the major dairy goats in China, and their milk is one of the major milk supplies for the Chinese dairy industry. To explore the whey proteome of Xinong Saanen goat milk, we analyzed the whey proteins of goat colostrum and mature milk using proteomic techniques. We identified a total of 314 and 524 proteins in goat colostrum and mature milk, respectively. Our data showed the first 3 functional categories of signal, disulfide bond, and secreted in both milk types. The most abundant Gene Ontology annotations in both milks were the biological process of positive regulation of extracellular regulated protein kinases (ERK)1 and ERK2 cascade; the cellular component of extracellular exosome; and the molecular function of calcium ion binding. Goat colostrum whey proteins showed more disease-related pathways, and mature milk showed more pathways associated with metabolism. Moreover, we observed several pathways involved in intestinal mucosal immunity only in colostrum. Protein–protein interaction network and module analysis revealed that complement and coagulation cascades and *Staphylococcus aureus* infection were significant in the whey proteins of both milks, and carbon metabolism was more common for mature milk than for colostrum. These findings could provide useful information for the use of goat milk whey proteins in the Chinese dairy industry.

Key words: proteomics, whey protein, Xinong Saanen goat, colostrum, mature milk

INTRODUCTION

Goat milk has been widely used in the dairy industry due to its importance for health (Park and Guo, 2008). Rich nutrients are presented in goat milk, including

protein, fat, lactose, vitamins, and minerals, of which protein is a key component. Goat milk proteins consist of 70% casein, 25% whey protein, and 5% milk fat globule membrane protein (Chen et al., 2019). Although the proportion of whey protein is not the highest, it is known to have a wide range of biological activities. Whey protein contains a broad spectrum of antibacterial properties and can act as an antibacterial/viral agent (Beljaars et al., 2004; Chaneton et al., 2011). Passive immunization (Saint-Sauveur et al., 2008) and the balance of intestinal flora in infants are also influenced by whey protein (Schmelzle et al., 2003).

Studies of the nutritional and functional properties of milk proteins have been carried out for decades. In recent years, more studies have explored milk proteins, especially some low-abundance proteins, using proteomic techniques. Of these, several studies on the whey proteome of goat milk have been reported. Lu et al. (2018) reported 213 proteins in the serum of goat tank milk and indicated a high level of complement proteins in goat milk serum. Our previous study found 283 whey proteins from the mature milk of Guanzhong goats using proteomics technology (Sun et al., 2019). Anagnostopoulos et al. (2016) identified 486 and 595 proteins in the milk of 2 indigenous Greek goat breeds using SDS-PAGE and liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). In another study, a total of 452 proteins were found in Camosciata goat milk by coupling combinatorial peptide ligand library technology, SDS-PAGE, and nano-LC-MS/MS (Cunsolo et al., 2015). Milk protein components differ significantly depending on the stage of lactation (Kirovski et al., 2014). Studies on whey proteomes at different stages of lactation have been reported in human (Liao et al., 2011), bovine (Yang et al., 2017), and yak milk (Yang et al., 2015). Several previous studies also focused on whey proteomes of goat milk at different stages of lactation (Hernández-Castellano et al., 2014a,b, 2015, 2016).

Xinong Saanen goat milk is one of the major milk supplies in China. Whey proteins play an essential role

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in nutrition and protection for kids. The objectives of this study were to characterize and compare whey proteomes in the colostrum and mature milk of Xinong Saanen goats using LC-MS/MS; we also discussed the differences in biological properties between goat colostrum and mature milk.

MATERIALS AND METHODS

Materials

Raw milk samples were collected from 30 Xinong Saanen goats from Lvnengshengtai Dairy Co. Ltd. (Baoji, Shanxi, China). Colostrum and mature milk were from the first batch of milk in the morning, collected from the same batch of healthy goats after 0 to 7 d and 1 mo postpartum, respectively. After sampling, all milk samples were frozen and taken immediately to the laboratory, where they were stored at -80°C until analysis.

Trypsin (sequencing grade) was obtained from Promega Corporation (Madison, WI). Protease inhibitor cocktail tablets were obtained from Roche (Mannheim, Germany). An ultra-centrifugal filter (10-kDa cutoff) was obtained from Sartorius (Göttingen, Germany). A Micro BCA protein assay kit and Pierce C18 tips were obtained from Thermo Fisher Scientific (Rockford, IL). Trifluoroacetic acid, dithiothreitol, iodoacetamide, methanol, acetonitrile, formic acid, and all other chemicals were of analytical grade and obtained from Sigma-Aldrich Corporation (St. Louis, MO). All aqueous solutions were prepared using Milli-Q treated water.

Preparation of Whey Proteins

We carried out the preparation of whey proteins according to the method of our previous study (Sun et al., 2019) with some modifications. All 30 colostrum samples were mixed individually. Then, 50 mL of the mixed milk was added a protease inhibitor cocktail (Roche) and incubated with slow agitation at room temperature for 30 min. After that, the sample was centrifuged at $8,000 \times g$ at 4°C for 20 min to obtain solid fat and skim milk. After the upper fat layer was removed, the skim milk was centrifuged at $50,000 \times g$ at 4°C for 2 h. Whey proteins were in the liquid phase. The concentration of whey proteins was quantified using the BCA protein assay kit. Mature milk samples were pretreated in the same way as the same as colostrum.

Trypsin Digestion

We performed a filter-aided sample preparation procedure for protein digestion. A solution containing 100

μg of whey proteins was mixed with dithiothreitol (final concentration of 10 mM) and kept at 56°C for 30 min. After the mixture was cooled to room temperature, iodoacetamide was added to a final concentration of 60 mM and incubated at room temperature for 30 min in the dark. The incubated sample was then loaded onto a filter (10 kDa cutoff) and centrifuged at $14,000 \times g$ for 15 min. Then, the filter was washed twice using 300 μL of buffer (8 M urea in 100 mM Tris/HCl; pH 8.5). After that, 200 μL of 50 mM NH_4HCO_3 solution was added to the filter and centrifuged at $14,000 \times g$ for 15 min. This step was repeated twice. Finally, 1.25 μg of trypsin was added and the mixture incubated at 37°C overnight. The digestion procedure was stopped by adding formic acid (10%), and digested peptides were collected by centrifuging at $14,000 \times g$ for 10 min. After desalting with the C18 tips, the collected peptides were vacuum-dried and stored at -20°C until analysis.

LC-MS/MS Analysis

We used the Easy-nLC 1000 system coupled with a Q Exactive mass spectrometer (both Thermo Fisher Scientific) for LC-MS/MS analysis, according to the method reported previously (Sun et al., 2019). Dried samples were re-suspended with mobile phase A (0.1% formic acid in water). Then, samples were loaded onto a pre-column ($100 \mu\text{m} \times 2 \text{ cm}$; $5 \mu\text{m}$; C18 particles) and separated by linear gradient elution with mobile phase B (0.1% formic acid in 80% acetonitrile) on an analytical column ($75 \mu\text{m} \times 100 \text{ nm}$; $3 \mu\text{m}$; C18 particles) at a flow rate of 300 nL/min. The MS conditions were as follows: spray voltage was 1.8 kV and capillary temperature was 300°C . Mass scans were acquired at a resolving power of 70,000 with a maximum ion injection time of 50 ms, and the scan range was 350 to 2,000 mass/charge (m/z). The top 15 most abundant precursor ions were chosen for higher-energy collision dissociation fragmentation with normalized collision energies of 27 eV. The resolution for higher-energy collision dissociation spectra was set at 17,500, the scan range at 200 to 2,000 m/z , and the maximum ion injection time at 45 ms. The dynamic exclusion was 60 s.

Data Analysis

We analyzed all LC-MS/MS raw data files using Proteome Discoverer 2.2 software (version 2.2; Thermo Fisher Scientific). Because of insufficient protein annotation for goat species, we searched data under a "Ruminantia" database (Cunsolo et al., 2015; Anagnostopoulos et al., 2016) downloaded from UniProt (Swissprot and trEMBL; <https://www.uniprot.org/>). We included N-terminal acetylation, methionine, and

phosphorylation of serine and threonine as variable modifications.

We classified all identified proteins using the online Database for Annotation, Visualization and Integrated Discovery (<http://david.abcc.ncifcrf.gov/home.jsp>) according to functional categories (UP_KEYWORDS), Gene Ontology (GO) annotations and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. We performed protein-protein interaction (PPI) network and module analysis using the online Search Tool for the Retrieval of Interacting Genes (<http://string-db.org>) and Cytoscape software (Shannon et al., 2003).

RESULTS AND DISCUSSION

Identification of Whey Proteins

We identified a total of 643 whey proteins in goat colostrum and mature milk. Among them, 195 proteins were common for both, and 119 and 329 proteins were unique for colostrum and mature milk, respectively (Figure 1). In previous studies of whey proteins in goat milk, 213 to 595 proteins have been found using proteomics techniques (Cunsolo et al., 2015; Anagnostopoulos et al., 2016; Lu et al., 2018; Sun et al., 2019). Previously identified whey proteins in goat milk include complement proteins (e.g., C3, C4), anti-proteases (e.g., SERPINA1, SERPINA3–6) and biglycan. Complement proteins are closely related to immune response; anti-proteases can protect milk proteins such as immunoglobulins from gastrointestinal digestion so that they can exert their biological effects (Chowanadisai and Lonnerdal, 2002; Beck et al., 2015); biglycan is a species-specific marker for goat milk (Yang et al., 2013).

Functional Categories of the Identified Whey Proteins

All identified whey proteins from goat colostrum and mature milk were classified based on their “UP_KEYWORDS”; the top 10 functional categories are shown in Figure 2. Whey proteins in both milk types showed the same first 3 categories: signal, disulfide bond, and secreted. The percentages of proteins involved in signal, disulfide bond and secreted were 40.76, 18.48, and 10.33%, respectively, in colostrum, and 30.72, 12.95, and 7.23%, respectively, in mature milk. Compared with mature milk, colostrum showed more whey proteins related to serine protease (2.72%) and innate immunity (1.63%). This may be because in addition to providing nutrition for newborn mammals, colostrum also provides a non-negligible immune function. Serine

proteases play an important role in mammalian digestion, coagulation, and complement systems. Innate immunity is the body’s natural immune defense function, and the basis of all immune responses. More whey proteins related to glycolysis (1.51%), acetylation (2.41%), and oxidoreductase (3.01%) were found in mature milk, closely associated with metabolism.

GO Analysis of the Identified Whey Proteins

We annotated all identified whey proteins with GO functions and classified them according to biological process, cellular component, and molecular function (Figure 3). The whey proteins in both milks were involved primarily in the biological process of positive regulation of extracellular regulated protein kinase 1 (ERK1) and ERK2 cascade; the cellular component of extracellular exosome; and the molecular function of calcium ion binding.

As shown in Figure 3a, the most common biological process in whey proteins from colostrum was positive regulation of ERK1 and ERK2 cascade (3.26%); innate immune response, blood coagulation, and fibrinolysis ranked second, all with 2.71%. For mature milk, the biological processes of positive regulation of ERK1 and ERK2 cascade, carbohydrate metabolic process, and cell redox homeostasis ranked first, all involving 2.71% of whey proteins, followed by innate immune response, with 2.41%. Our previous study showed that positive regulation of ERK1 and ERK2 cascade was also the most common biological process of whey proteins in Guanzhong goat milk (Sun et al., 2019). We know that ERK can regulate a wide range of biological processes,

Figure 1. Venn diagram of whey proteins identified in goat colostrum and mature milk.

including cell proliferation, differentiation, and apoptosis, and it can play a key role in behavioral responses and cognition, including learning and memory. Both coagulation and fibrinolysis are involved in metabolic regulation. Certain secretions in mammals, such as milk, contain activins of plasminogen, which can activate fibrinolysis and keep the secretion pipeline unobstructed.

Regarding cellular component (Figure 3b), a large portion of whey proteins in colostrum and mature milk were related to the extracellular exosome and extracellular space. In total, these 2 categories accounted for 33.15 and 21.74% of proteins in colostrum and 37.65 and 17.78% of proteins in mature milk, respectively.

Figure 3c shows the classification results of whey proteins based on molecular function. Calcium ion binding was the most abundant function of whey proteins from both colostrum and mature milk, accounting for 5.98 and 3.92%, respectively. The first 4 functions of whey proteins in both milk types were the same: calcium ion

binding > serine-type endopeptidase inhibitor activity > serine-type endopeptidase activity > glycoprotein binding. Similar results have been reported in previous studies; for example, calcium ion binding was also one of the main molecule functions in whey proteins of human, bovine, and yak milk (Lu et al., 2018; Sun et al., 2019).

KEGG Pathway Analysis of the Identified Whey Proteins

We performed KEGG pathway analysis of all identified whey proteins as shown in Table 1. We obtained a total of 35 KEGG pathways; most whey proteins in colostrum (36.4%) fell under disease-related pathways (ranked second in mature milk at 22.3%); most whey proteins in mature milk fell under metabolism-related pathways (25.6%).

Goat colostrum showed a larger portion of whey proteins and more disease-related pathways than mature milk. However, the pathways for *Staphylococcus aureus* infection, pertussis, and systemic lupus erythematosus ranked first in the whey proteins of both milk types. The percentages of whey proteins associated with these 3 pathways were same, at 5.4% for colostrum and 3.6% for mature milk. Unlike in colostrum, in mature milk a large number of whey proteins fell under metabolism-related pathways, of which biosynthesis of antibiotics (5.4%), carbon metabolism (4.8%), biosynthesis of amino acids (2.7%), and glycolysis/gluconeogenesis (2.4%) were the first 4 categories. Unexpectedly in this subcategory, only 1.1% of whey proteins from colostrum fell under the carbon metabolism pathway. Previously, a high number of proteins in human and ruminant milk serum were found to be related to metabolic processes (Lu et al., 2018). In another study, metabolism-related pathways (such as glycolysis/gluconeogenesis and biosynthesis of amino acids) also enriched many differentially expressed whey proteins in human and bovine colostrum and mature milk (Yang et al., 2017).

Whey proteins in both milks also showed a high portion of proteins involved in complement and coagulation cascades (a subcategory of immune system). The complement and coagulation cascades pathway plays an important role in maintaining the balance of the coagulation–fibrinolytic system and in activating innate immunity (Keragala et al., 2018), and the associated proteins are critical for the health and nutrition of goat kids (Moreno-Indias et al., 2012a,b). Of note, we found the intestinal immune network for IgA production and the transforming growth factor- β (TGF- β) signaling pathway only in colostrum. Immunoglobulin A is the most abundant antibody subtype, produced

Figure 2. Top 10 functional categories of whey proteins identified in (a) goat colostrum, and (b) mature milk based on “UP_KEYWORDS” (online DAVID tool, <https://david.ncifcrf.gov/home.jsp>).

Figure 3. Gene Ontology classification of whey proteins identified in goat colostrum and mature milk based on (a) biological process, (b) cellular components, and (c) molecular function.

mainly in the intestine, and a key mediator in intestinal mucosal immunity (Pabst, 2012; Slack et al., 2012). Previous studies indicated that IgA played an essential role in the regulation of gut microbiota and immune homeostasis (Peterson et al., 2007; Kato et al., 2014). Neutrophil activation and migration induced by IgA may be closely related to inflammatory response and severe tissue damage in ulcerative colitis (Kaser et al., 2010; Palm et al., 2014). As a cytokine, TGF- β can be involved in the production of intestinal secretory IgA and induce IgA class switching of B cells (Stavnezer and Kang, 2009).

PPI Network and Module Analysis of the Identified Whey Proteins

We built the PPI networks of whey proteins identified in goat colostrum and mature milk and analyzed the top 3 modules as shown in Figures 4 and 5 and Tables 2 and 3. Whey proteins from both milk types

showed that the most significant modules were related to immunity, and the main functions of the other 2 modules were different, a finding that may reveal the different roles played by whey proteins from colostrum and mature milk.

Figure 4a shows a PPI network of colostrum whey proteins that contained 118 nodes and 327 edges, with a PPI enrichment P -value $<1.0E-16$. Among the 118 nodes, we identified 9 central nodes with a degree >20 (i.e., each node had more than 20 connections/interactions) were identified, including plasminogen (PLG), serpin family C member 1 (SERPINC1), GC vitamin D binding protein (GC), α -1-microglobulin/bikunin precursor (AMBP), apolipoprotein A1 (APOA1), fibrinogen gamma chain (FGG), coagulation factor II/thrombin (F2), α 2-HS glycoprotein (AHSG) and hemopexin (HPX). The greater the degree value of a node, the higher the centrality of the node, and the more important it was in the network. We selected the 3 modules with the most intensive interactions in the

Table 1. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of whey proteins from goat colostrum and mature milk¹

KEGG pathway name	Colostrum			Mature milk		
	Count	%	Fold enrichment	Count	%	Fold enrichment
Complement and coagulation cascades	21	11.4	32.1	26	7.8	19.6
<i>Staphylococcus aureus</i> infection	10	5.4	17.0	12	3.6	10.0
Pertussis	10	5.4	14.6	12	3.6	8.6
Systemic lupus erythematosus	10	5.4	7.9	12	3.6	4.7
Prion diseases	4	2.2	16.1	6	1.8	11.9
Viral carcinogenesis	7	3.8	3.8	9	2.7	2.4
Legionellosis	4	2.2	7.2	9	2.7	8.0
Tuberculosis	6	3.3	3.7	8	2.4	2.4
Leishmaniasis	4	2.2	5.8	—	—	—
ECMA-receptor interaction	4	2.2	5.4	5	1.5	3.3
Lysosome	4	2.2	3.8	6	1.8	2.8
Phagosome	5	2.7	3.4	11	3.3	3.7
Chagas disease (American trypanosomiasis)	4	2.2	4.2	—	—	—
Herpes simplex infection	5	2.7	2.7	—	—	—
Viral myocarditis	3	1.6	4.6	—	—	—
Mineral absorption	2	1.1	5.6	—	—	—
Intestinal immune network for IgA production	2	1.1	4.2	—	—	—
Thyroid hormone synthesis	2	1.1	3.5	—	—	—
TGF- β signaling pathway	2	1.1	3.0	—	—	—
Carbon metabolism	2	1.1	2.1	16	4.8	8.2
Biosynthesis of antibiotics	—	—	—	18	5.4	4.9
Biosynthesis of amino acids	—	—	—	9	2.7	7.4
Protein processing in endoplasmic reticulum	4	2.2	2.8	13	3.9	4.5
Glycolysis/gluconeogenesis	—	—	—	8	2.4	7.2
Pentose phosphate pathway	—	—	—	5	1.5	10.3
Starch and sucrose metabolism	—	—	—	5	1.5	9.6
Salmonella infection	—	—	—	6	1.8	4.1
Galactose metabolism	—	—	—	4	1.2	7.7
Citrate cycle (TCA cycle)	—	—	—	4	1.2	7.0
Cysteine and methionine metabolism	—	—	—	4	1.2	5.7
Amino sugar and nucleotide sugar metabolism	—	—	—	4	1.2	4.7
Butirosin and neomycin biosynthesis	—	—	—	2	0.6	23.8
HIF-1 signaling pathway	2	1.1	2.4	5	1.5	3.0
Glyoxylate and dicarboxylate metabolism	—	—	—	3	0.9	5.9
Fructose and mannose metabolism	—	—	—	3	0.9	5.6

¹ECMA = extracellular matrix; HIF = hypoxia-inducible factor; TCA = tricarboxylic acid; TGF = transforming growth factor.

Figure 4. Protein-protein interaction (PPI) network and module analysis of whey proteins identified in goat colostrum. (a) PPI network; (b) top 3 modules from the PPI network. Each circle node represents 1 protein, the connection between node and node represents the interaction between 2 proteins, and lines with different colors indicate different interaction types.

a **b**

Module 1

Module 2

Module 3

Figure 5. Protein-protein interaction (PPI) network and module analysis of whey proteins identified in goat mature milk. (a) PPI network; (b) top 3 modules from the PPI network. Each circle node represents 1 protein, the connection between node and node represents the interaction between 2 proteins, and lines with different colors indicate different interaction types.

Table 2. Module analysis of the protein–protein interaction network of whey proteins identified in goat colostrum¹

Module	Function description	FDR ¹	Nodes ²
1	Complement and coagulation cascades	3.87E–14	A2M, F2, FGA, FGB, FGG, KNG1, PLG, SERPINC1
2	<i>Staphylococcus aureus</i> infection	1.84E–17	C2, C3, C5, CFI, ENSOARG00000000743, ENSOARG00000000751, ENSOARG00000005384
3	Extracellular matrix–receptor interaction	0.0101	CD44, THBS1

¹FDR = false discovery rate.²A2M = alpha-2-macroglobulin; F2 = coagulation factor II/thrombin; FGA = fibrinogen alpha chain; FGB = fibrinogen beta chain; FGG = fibrinogen gamma chain; KNG1 = kininogen 1, PLG = plasminogen; SERPINC1 = serpin family C member 1; C2 = complement component 2; C3 = complement component 3; C5 = complement component 5; CFI = complement factor I; ENSOARG00000000743 = anaphylatoxin-like domain-containing protein; ENSOARG00000000751 = A2M_N_2 domain-containing protein; ENSOARG00000005384 = NTR domain-containing protein; CD44 = CD44 molecule; THBS1 = thrombospondin 1.

PPI network, and analyzed the functional annotation of the proteins involved (Figure 4b, Table 2). The proteins in modules 1 to 3 were associated mainly with complement and coagulation cascades, *S. aureus* infection, and extracellular matrix–receptor interaction, respectively. Extracellular matrix plays an important role in tumor, fibrosis, wound healing, and inflammatory response (Raghow, 1994; Borg, 2004; Schultz and Wysocki, 2009; Petrey and de la Motte, 2017).

For whey proteins in mature milk, the PPI network contained 212 nodes, 766 edges, and a PPI enrichment *P*-value <1.0E–16 (Figure 5a). We identified more central nodes in mature milk than in colostrum. In addition to the 9 nodes in colostrum, we screened another 8 nodes as central nodes (degree >20): PLG, APOA1, SERPINC1, GAPDH, GC, HPX, FGG, F2, AHSG, AMBP, fibrinogen α chain (FGA), phosphoglycerate kinase 1 (PGK1), prolyl 4-hydroxylase subunit β (P4HB), fibrinogen β chain (FGB), glucose-6-phosphate isomerase (GPI), fetuin B (FETUB) and apolipoprotein A4 (APOA4). For functional annotation of proteins involved in top 3 modules (Figure 5b, Table 3), module 1 showed the most abundant pathway of complement and coagulation cascades; the most abundant pathway in module 2 was carbon metabolism. The main path-

way in module 3 was *S. aureus* infection, which ranked second in colostrum. The role of mature milk whey proteins in metabolism was not negligible compared with that of colostrum. In a previous study, a high portion of whey proteins related to metabolism were observed in human milk 15 d and 2 mo after delivery (Hahn et al., 2018). Additionally, the crucial roles of complement and coagulation cascades observed in goat colostrum and mature milk were agreement with previous results in whey proteomes of human, bovine, yak, and camel milk (Yang et al., 2013, 2015, 2017).

CONCLUSIONS

Using proteomics technology, we identified a total of 643 whey proteins in goat colostrum and mature milk (195 in both; 119 and 329 only in colostrum and mature milk, respectively). Whey proteins from goat colostrum were different from those in mature milk with respect to functional categories, GO annotations, KEGG pathways, and PPI networks. Immune-related proteins were abundant in both milk types. We found more disease-related proteins in colostrum, and more metabolism-related proteins in mature milk. These findings could provide important information for understanding the

Table 3. Module analysis of the protein–protein interaction network of whey proteins identified in goat mature milk

Module	Function description	FDR ¹	Nodes ²
1	Complement and coagulation cascades	1.26E–15	A2M, CPB2, F2, FGA, FGB, FGG, KNG1, PLG, SERPINC1
2	Carbon metabolism	4.81E–21	ALDOC, GAPDH, GPI, IDH1, MDH1, MDH2, PGD, PGK1, TALDO1, TKT
3	<i>Staphylococcus aureus</i> infection	1.84E–17	C2, C3, C5, ENSOARG00000000751, ENSOARG00000002722, ENSOARG00000002771, ENSOARG00000005384

¹FDR = false discovery rate.²A2M = alpha-2-macroglobulin; CPB2 = carboxypeptidase B2; F2 = coagulation factor II/thrombin; FGA = fibrinogen alpha chain; FGB = fibrinogen beta chain; FGG = fibrinogen gamma chain; KNG1 = kininogen 1, PLG = plasminogen; SERPINC1 = serpin family C member 1; ALDOC = aldolase, fructose-bisphosphate C; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; GPI = glucose-6-phosphate isomerase; IDH1 = isocitrate dehydrogenase (NADP(+)) 1; MDH1 = malate dehydrogenase 1; MDH2 = malate dehydrogenase 2; PGD = phosphogluconate dehydrogenase; PGK1 = phosphoglycerate kinase 1; TALDO1 = transaldolase 1; TKT = transketolase; C2 = complement component 2; C3 = complement component 3; C5 = complement component 5; ENSOARG00000000751 = A2M_N_2 domain-containing protein; ENSOARG00000002722 = complement component 4-like; ENSOARG00000002771 = anaphylatoxin-like domain-containing protein; ENSOARG00000005384 = NTR domain-containing protein.

composition and unique biological properties of goat milk at different stages of lactation.

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