UNIVERSIDADE FEDERAL DE VIÇOSA

LAVÍNIA MARTINS DA SILVA

COMPARAÇÃO DOS PERFIS PROTEÔMICOS DE COLOSTRO E DE LEITE MADURO DE CABRAS DA RAÇA SAANEN VISANDO APLICAÇÃO NA INDÚSTRIA LÁCTEA E NO REBANHO

VIÇOSA – MINAS GERAIS
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Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Bioquímica Aplicada, para obtenção do título de *Magister Scientiae*.

Orientadora: Maria Cristina Baracat Pereira

Coorientadores: José Domingos Guimarães
Edvaldo Barros

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Assentimento:

Lavínia Martins da Silva
Autora

Maria Cristina Baracat Pereira
Orientadora

A Deus, aos meus familiares e aos meus amigos.

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RESUMO

SILVA, Lavínia Martins, M.Sc., Universidade Federal de Viçosa, agosto de 2022. **Comparação dos perfis proteômicos de colostro e de leite maduro de cabras da raça Saanen visando aplicação na indústria láctea e no rebanho**. Orientadora: Maria Cristina Baracat Pereira. Coorientadores: José Domingos Guimarães e Edvaldo Barros.

A cabra da raça Saanen é uma das maiores produtoras de leite e possui um grande potencial proteico, ganhando visibilidade de empresas lácteas para produção de derivados. O objetivo deste trabalho foi comparar os perfis de proteínas do leite de cabra nas fases de lactação, conhecidas como colostro e leite maduro, por meio de análises por LC-MS/MS. Foram identificadas no total 228 proteínas, das quais 147 exclusivas do colostro, 18 exclusivas do maduro e 63 presentes em ambas as amostras. Das proteínas comuns identificadas entre as amostras de leite nas fases da lactação, a análise quantitativa mostrou 22 proteínas diferencialmente abundantes, sendo que 14 proteínas apresentaram níveis mais abundantes no colostro e oito, no maduro. A rede de interação entre as proteínas identificadas mostrou 11 clusters, sendo quatro maiores e sete menores, que são organizados em relação a processos biológicos de imunidade, energético e dobramento de proteínas. As informações obtidas direcionam estudos para a melhorar compreensão das funções das proteínas nas fases de lactação, com vistas à aplicação na indústria láctea e no rebanho.

Palavras-chave: Leite Caprino. Nutrição. Fases da lactação.

ABSTRACT

SILVA, Lavínia Martins, M.Sc., Universidade Federal de Viçosa, August 2022. **Comparison of the proteomic profiles of colostrum and mature milk from Saanen goats for application in the dairy industry and in the herd.** Adviser: Maria Cristina Baracat Pereira. Co-advisers: José Domingos Guimarães and Edvaldo Barros.

The Saanen goat is one of the largest milk producers and has a great protein potential, gaining visibility of dairy companies for the production of derivatives. The objective of this work was to compare the protein profiles of goat milk in the colostrum and mature milk lactation phases through LC-MS/MS analysis. A total of 228 proteins were identified, of which 147 were present only in colostrum, 18 only in mature and 63 in both samples. Of the common proteins identified among the milk samples in the phases of lactation, the quantitative analysis showed 22 differentially abundant proteins, with 14 proteins showing higher levels in colostrum and eight in the mature one. The interaction network between the identified proteins showed 11 clusters, four major and seven minor ones, which are organized in relation to biological processes of immunity, energetics and protein folding. The information obtained directs studies to improve the understanding of the functions of proteins in the phases of lactation, with a view to application in the dairy industry and in the herd.

Keywords: Goat Milk. Nutrition. Phases of lactation.

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1. INTRODUÇÃO

O leite de cabra é o mais produzido no mundo, mas o menos comercializado, ficando atrás do leite de vaca e de búfala. Além de ser rico em proteínas com grande valor biológico, os glóbulos de gordura são menores, facilitando sua absorção, e possui grande quantidade de triglicerídeos que ajudam a prevenir complicações cardíacas (KEBEDE, 2014)

O leite caprino apresenta características valiosas para a saúde humana. Possui mais minerais que o leite de vaca, como o cálcio, o magnésio e o cobre. A vitamina A também é encontrada em maior quantidade, e é valiosa para a saúde dos olhos e pele dos seres humanos. O leite de cabra possui um menor potencial alergênico do que o leite bovino, por possuir uma menor fração proteica da alfa-S1 caseína (APARNATHI et al., 2017).

Para os animais, durante a gestação do caprino, não ocorre a passagem de anticorpos da mãe para o animal, então o recém-nascido precisa da imunidade fornecido pelo alimento para a sua sobrevivência. Os anticorpos são adquiridos do colostro, que é o leite secretado nos primeiros dias pós-parto, que é rico em imunoglobulinas e garante que o cabrito se desenvolva de forma saudável.

O aproveitamento inteligente do leite de cabra para gerar efeitos econômicos satisfatórios poderia ser pela produção de leite pasteurizado, UHT, leite em pó, queijos finos, iogurte, bebidas lácteas, doces ou outros produtos. Além do setor alimentício, o leite de cabra pode ser usado como matéria-prima para a indústria de cosméticos, para a geração de produtos para cuidados com o cabelo e a pele, em função do seu elevado potencial biológico, originalmente natural e sem toxicidade (KAZIMIERSKA; KALINOWSKA-LIS, 2021).

A raça Saanen é originária da Suíça, e é conhecida como a maior produtora de leite do mundo. No Brasil, os maiores rebanhos estão na região Nordeste, e a maior produção de leite está no Sudeste. Em função da grande produção leiteira caprina e do aumento do uso do leite de cabra como matéria prima para fins nobres, ao lado do aspecto nutricional para os recém-nascidos, o objetivo deste trabalho foi caracterizar e comparar o perfil de proteínas do leite colostro e maduro utilizando a técnica LC/MS-MS e abordar suas características biológicas nestas fases de lactação, visando informações para a criação de formulações para cabritos recém-nascidos que não têm acesso ao colostro, ao lado da aplicação deste conhecimento na indústria láctea, para expandir o mercado de produtos derivados do leite caprino.

2. OBJETIVOS

2.1. Geral

Avaliar os perfis de proteínas do leite de cabra em duas fases de lactação, colostro e leite maduro para melhor compreensão das funções das proteínas, visando a posterior aplicação na indústria láctea e no rebanho.

2.2. Específicos

a) Realizar a análise proteômica por LC-MS/MS para caracterizar o perfil proteico geral do leite caprino;

b) Realizar a análise proteômica diferencial, por *Label Free Proteomics*, para caracterizar as proteínas diferencialmente abundantes do leite de cabra nas fases de colostro e maduro;

c) Determinar a classificação funcional das proteínas do leite caprino em amostras de acordo com a fase de lactação, com vistas a identificar aplicações na indústria láctea e no rebanho.

3. REVISÃO BIBLIOGRÁFICA

3.1. Cabra

Antigamente, a cabra era conhecida como a “vaca de pobre”, considerada na história da humanidade como o animal mais antigo usado para domesticação. Dado o baixo valor econômico necessário para a sua criação, esse animal é tido como uma fonte de leite e carne mais baratos, quando comparados ao bovino (UTAAKER, 2021).

As cabras têm um desempenho satisfatório em regiões de clima tropical, subtropical, desértico e mediterrâneo, isso porque possui a capacidade de se adaptar às mudanças climáticas revelando estratégias adaptativas (SILANIKOVE; KOLUMAN, 2015). Por isso, mais de dois terços das cabras no mundo estão localizadas em países subtropicais e tropicais.

Segundo a *Food and Agriculture Organization* (*FAO*), mundialmente, o número total de cabras está perto de 1 bilhão, e 90% da população total das cabras está concentrada nos países em desenvolvimento. A Ásia domina o ranking de maior criação de cabras, logo na sequência, a África (NAYIK, 2021).

De acordo com a Empresa Brasileira de Pesquisa Agropecuária (Embrapa), o rebanho caprino brasileiro foi previsto em 12,1 milhões de cabeças em 2020, com aumento de 4,0% comparado ao ano de 2019. A região Nordeste possui o maior rebanho do país, totalizando 11,49 milhões de cabeças em 2020, correspondente a 95% do rebanho nacional, comprovando a adaptação desses animais às condições climáticas do semiárido, em particular no bioma da Caatinga (ARAGÃO MAGALHÃES, 2021).

Nos países dos continentes de baixa renda, como África, Ásia e América Latina, as cabras são criadas para o consumo da carne e do leite e frequentemente é a única fonte de proteínas para as crianças. Geralmente, os criadores das cabras são de classe social mais baixa e na região rural, são manuseadas por crianças e mulheres (UTAAKER, 2021).

A criação de cabras não está apenas associada a produtores de baixa renda. Os preços atraentes dos produtos de cabra, em especial derivados do leite, ocasionou a adesão de novos produtores e investidores para o mercado. O queijo de cabra de boa qualidade está associado à França, mas outros países também o produzem, como os Estados Unidos, a Itália e a Espanha. O desenvolvimento da indústria do leite de cabra em países mais pobres é propício, e para isso é necessário acesso à informação de qualidade, a produção de matéria-prima e de tecnologia, além de políticas de apoio (MILLER; LU, 2019)

3.2. Leite de cabra

O leite de cabra é caracterizado como um líquido branco, viscoso, com odor específico. A composição química básica do leite de cabra contém, em média, 13,2% de sólidos totais, sendo 4,5% de gordura, 3,6% de proteína, 4,3% de lactose e 0,8% de minerais (TURKMEN, 2017).

Em relação à composição nutricional, o leite de cabra possui grande proporção de ácidos graxos de cadeia pequena e média, tais como: ácidos butírico, capróico, caprílico, cáprico, láurico, mirístico, palmítico e linolênico. Por predominar no leite de cabra, três desses (ácidos capróico, caprílico e cáprico) possuem o nome de cabras, dos quais, os ácidos capróico e caprílico mostram ter atividade antimicrobiana. O ácido linoleico conjugado (CLA) presente no leite de cabra diminui o estresse oxidativo, aterosclerose e melhora o nível de lipídios no sangue. Além disso, garante proteção contra o crescimento de tumores na glândula mamária e na pele (SILANIKOVE, 2010).

O leite caprino também possui uma alta concentração de triglicerídeos de cadeias médias, facilitando a absorção de nutrientes e a produção de energia para o corpo. Além disso, possuem a capacidade de inibir e/ou limitar a deposição de colesterol nos tecidos (KEBEDE, 2014), o que faz com que auxilie na prevenção de aterosclerose, ataques cardíacos, derrames e outras complicações cardíacas. Por causa da grande concentração de potássio, o leite de cabra diminui a pressão arterial (APARNATHI et al., 2017).

O principal carboidrato do leite de cabra é a lactose, que é produzida nas glândulas mamárias e serve como fonte de energia, além de facilitar a absorção de cálcio, fosfato, manganês e magnésio, e ajudar no desenvolvimento das bactérias intestinais Gram-positivas, evitando o crescimento populacional de microrganismos patogênicos no hospedeiro (UGIDOS-RODRÍGUEZ; MATALLANA-GONZÁLEZ; SÁNCHEZ-MATA, 2018). Outros carboidratos como glicoproteínas, glicopeptídeos e oligossacarídeos, além de nucleotídeos, também são encontrados, mas em menores quantidades. Os oligossacarídeos do leite de cabra mostraram reduzir a inflamação intestinal e ajudar na melhora da colite (inflamação do cólon) em animais (DADDAOUA et al., 2006).

Acerca dos minerais presentes no leite de cabra em maiores concentrações do que o leite de vaca (SHI; LI, 2021), cita-se o cálcio, que é responsável por fornecer força aos ossos, os quais sustentam a locomoção e servem como reservatórios para manter os níveis séricos desse

mineral (WEAVER; PEACOCK, 2011). O magnésio é fundamental para o metabolismo do trifosfato de adenosina (ATP), síntese de DNA e RNA, reprodução e síntese de proteínas. Além do mais, ele regula a contração muscular, pressão arterial, excitabilidade cardíaca, transmissão nervosa, condução neuromuscular, metabolismo da insulina e tônus vasomotor (GRÖBER; SCHMIDT; KISTERS, 2015). O cobre é um cofator importante e/ou componente estrutural de várias enzimas de plantas e de animais. Geralmente, essas enzimas estão envolvidas em reações de óxido-redução, em função do seu alto potencial redox para Cu^{2+}/Cu^{+} (SCHEIBER; DRINGEN; MERCER, 2013).

A vitamina A é a que possui a maior diferença entre as outras vitaminas do leite de cabra e do de vaca. Isso porque a cabra converte todo o β-caroteno em vitamina A no leite e assim, o leite de cabra é mais branco e tem mais vitamina A do que o leite de vaca (TURKMEN, 2017). Essa vitamina é importante para a imunidade inata e a adaptativa, englobando imunidade permeada por células e respostas de anticorpos (KEBEDE, 2014).

3.2.1. Proteínas do leite de cabra

Os primeiros a discorrer sobre a composição do leite colostro de cabras, sobre as globulinas em especial, foram Bergman e Turner (1937), que reportaram que a proteína total era composta por 4 grupos de proteínas: caseína, albumina, globulina e caseína globulina (BERGMAN; TURNER, 1937).

Com o avanço das pesquisas, outras proteínas foram descobertas, e foram acomodadas em três grupos de proteínas de leite de cabra. O primeiro é o grupo da caseína (parte coagulável), principal proteína do leite e que possui os tipos: α-s1, α-s2, β, κ e γ caseínas. O segundo grupo envolve as proteínas do soro do leite, representadas pela alfa-lactoalbumina (α-La), beta-lactoglobulina (β-LG), imunoglobulinas IgG, IgM, albumina sérica, lactoferrina e lisozima (BIADAŁA; KONIECZNY, 2018). E por último, as proteínas das membranas dos glóbulos de gordura. As proteínas principais desse grupo são as mucinas, adipofilina, butirofilina e xantina oxidorredutase (GOULDING; FOX; O'MAHONY, 2020).

3.2.1.1. Principais Proteínas do Leite de Cabra

3.2.1.1.1. Caseína

A caseína faz parte da família das proteínas do leite que possuem resíduos de fósforo (fosfoproteína) e açúcar (glicoproteína), e no leite de cabra são encontradas quatro frações: β-caseína (fração dominante), α-s1 caseína, α-s2-caseína e κ-caseína (TAMIME et al., 2011). Devido às fosforilações encontradas nas caseínas, essas proteínas são responsáveis pela regulação do metabolismo do fosfato e do cálcio. Suas frações possuem a capacidade de se transformar em moléculas biologicamente ativas. Como exemplo, podemos citar a alfa-caseína, a qual se transforma em opioide, a casomorfina e as alfa- e beta-caseínas, as quais são precursoras de imunipeptídeos (KAZIMIERSKA; KALINOWSKA-LIS, 2021).

No leite de vaca, a alfa-s1-caseína é encontrada em maior quantidade e é responsável por provocar reações alérgicas. Normalmente, a proporção dessa proteína no leite de cabra é menor e, no caso de algumas raças, é ausente. Isso faz com que o leite de cabra se torne uma boa opção para substituir o leite de vaca para as pessoas que possuem alergia ou intolerância (BIADAŁA; KONIECZNY, 2018).

3.2.1.1.2. Alfa-lactoalbumina (α-La)

A alfa-lactoalbumina é uma proteína globular, uma albumina hidrofílica, proteína em maior concentração no leite. Ela possui aminoácidos essenciais como triptofano, cisteína e lisina. A cisteína na estrutura da proteína está envolvida no fortalecimento do sistema imunológico, ao passo que os elevados níveis de triptofano contribuem para a melhora do sono, humor e desenvolvimento cognitivo dos recém-nascidos.

Ao longo da lactação, as glândulas mamárias fornecem α-La e galactosiltransferase que, quando se juntam, compõem o complexo enzimático lactose sintase, que catalisa a síntese de lactose usando glicose e galactose (ALMEIDA et al., 2021).

3.2.1.1.3. Beta-lactoglobulina (β-Lg)

A beta-lactoglobulina é a uma proteína globular e faz parte da superfamília das lipocalinas, caracterizadas como proteínas de transporte com uma estrutura secundária característica, com nove fitas β antiparalelas (β–A a β–I) e uma α–hélice (VARLAMOVA; ZARIPOV, 2020).

Em função da presença de aminoácidos contendo enxofre e metionina, a molécula de β-Lg possui atividade antioxidante no leite e demonstra efeitos antitumorais. A β-Lg inibe a

adesão de patógenos às superfícies, não permitindo sua invasão. Assim ela possui atividade contra bactérias Gram-positivas, como *Bacillus subtilis* e *Staphylococcus aureus*, e Gram-negativas, como *Escherichia coli* e *Bordetella bronchiseptica* (KAZIMIERSKA; KALINOWSKA-IS, 2021).

3.2.1.1.4. Lactoferrina

A lactoferrina é uma glicoproteína de transferrina multifuncional que se liga ao ferro e é secretada por alguns fluidos, principalmente no leite (RAHMAN et al., 2009). Possui função antimicrobiana direta, pois não permite a proliferação e adesão dos microrganismos e/ou os mata. Isso só é possível pois tem habilidade de retirar ferro dos fluidos biológicos e desestabilizar as membranas dos microrganismos. Além disso, a lactoferrina é capaz de modular a resposta imune e tem um papel contra o choque séptico (SIQUEIROS-CENDÓN et al., 2014).

3.2.1.1.5. Imunoglobulinas

As imunoglobulinas, conhecidas como anticorpos, pertencem a uma família de proteínas com função de bioatividade de proteção. Elas são divididas em diversas classes, como por exemplo IgD, IgM, IgA, IgE e IgG. As principais classes das imunoglobulinas das secreções mamárias são IgG, IgA e IgM (HURLEY; THEIL, 2011).

As Igs são os principais a promover a proteção da mucosa intestinal contra microrganismos patogênicos, e no colostro são responsáveis por garantir a imunidade passiva ao neonato ruminante até que seu sistema imunológico esteja desenvolvido (GAPPER et al., 2007).

3.3. Fases de lactação

3.3.1. Colostro

O colostro é caracterizado como o leite que começa a ser produzido nas glândulas mamárias antes do nascimento do filhote e dura de três a cinco dias pós parto, e é diferente em volume, aparência e composição. O colostro é rico em nutrientes, e principalmente as proteínas lactoferrina e imunoglobulina (IgA). Oferece proteção ao recém-nascido por meio de imunidade passiva contra patógenos. O colostro possui pouca gordura e lactose, mostrando que

suas funções principais são imunológicas e tróficas. O nível de nutrientes e bioativos são achados em concentrações elevadas durante os primeiros dias e com o passar do tempo, esses níveis são normalizados (SANGWAN, 2021)

3.3.2. Maduro

O leite maduro é classificado como o leite secretado duas semanas pós parto. Possui aproximadamente 71 kcal/100 mL (CZOSNYKOWSKA-ŁUKACKA; KRÓLAK-OLEJNIK; ORCZYK-PAWIŁOWICZ, 2018), possui algumas características do leite colostro, mas possui um menor nível de proteínas e maior concentração de lactose e gordura. Em humanos, ocorre uma perda do epitélio mamário e, consequentemente, a relação sódio-potássio diminui e a concentração de lactose aumenta. Como o recém-nascido precisa de um volume maior de leite para seu crescimento, ocorre um aumento da produção láctea (BALLARD; MORROW, 2013; GIDREWICZ; FENTON, 2014).

3.4. A importância do colostro nos primeiros dias de vida para os caprinos

Nos ruminantes de pequeno porte, bovinos, equinos e suínos, a placenta não permite a passagem de imunoglobulinas da mãe para o feto ao longo da gestação, como ocorre em outras espécies animais. Por conseguinte, os cabritos precisam ingerir imunoglobulinas por meio da ingestão do colostro materno, adquirindo dessa forma, imunidade passiva e se protegendo contra infecções no início da vida. Caso o colostro não seja fornecido em quantidade ou qualidade ideais nas primeiras horas de vida, o cabrito sofre o risco de falha na transferência imune passiva, tendo como consequência o aumento da morbidade e mortalidade (KESSLER; BRUCKMAIER; GROSS, 2019).

Os animais que se desenvolvem em sistema de criação artificial devem ser alimentados, via mamadeira, com uma quantidade suficiente de colostro durantes seus primeiros dias de vida, para adquirir a imunidade passiva e aumentar sua produtividade futura (HERNÁNDEZ-CASTELLANO et al., 2015).

3.5. Produtos derivados do leite de cabra

O leite de cabra é considerado um alimento funcional pois possui compostos bioativos, como proteínas do soro do leite e caseína, que são essenciais para funções bioquímicas e fisiológicas, com influência na saúde e no metabolismo humano. Atualmente, em função da sua composição, o leite de cabra é usado como matéria-prima para produção de alimentos para crianças, idosos e pessoas com necessidades dietéticas especiais.

Em função do seu alto poder nutricional e digestibilidade, o leite de cabra vem se tornando fundamental para a dieta humana (NAYIK, 2021). Pode ser usado para a produção de leite tipo *ultra high temperature* (UHT, submetido a altas temperaturas e tempo curto), bebidas lácteas, produtos fermentados (iogurte e queijo), manteiga, sorvetes, doces, produtos condensados, e outros. E possui um grande potencial para o desenvolvimento de novos produtos e alimentos funcionais, capazes de promover a saúde (VERRUCK; DANTAS; PRUDENCIO, 2019).

3.6. Raça Saanen

A raça caprina Saanen é original da Suíça, possui o maior valor leiteiro do mercado e é a maior produtora de leite do mundo. Uma cabra Saanen chega a produzir 2,5 a 4,9 kg de leite por dia, com 3,0 a 3,5% de gordura (PESMEN; YARDIMCI, 2008). As cabras Saanen têm como características pêlos brancos e curtos, são de grande porte, orelhas pequenas, cascos amarelo-claros e pele rosé (Figura 1). As fêmeas da raça possuem peso por volta de 45 a 60 kg (CUNHA et al., 2004) e altura média de 70 a 83 cm. Já os machos, possuem o peso corporal entre 70 e 90 kg e altura de 80 a 95 cm (VIANA, 2017).

Figura 1: Raça Saanen

Fonte: PEREIRA, Carolina Rodrigues, 2018

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COMPARISON OF PROTEOMIC PROFILES OF COLOSTRUM AND MATURE MILK OF GOATS OF THE SAANEN BREED FOR APPLICATION IN THE DAIRY INDUSTRY AND IN THE HERD

ABSTRACT

The Saanen goat is one of the largest milk producers and has a great protein potential, gaining visibility of dairy companies for the production of derivatives. Our objective in this work was to compare the protein profiles of goat milk in the colostrum and mature milk lactation phases through LC-MS/MS analysis. A total of 228 proteins were identified, of which 147 were identified in colostrum, 18 in mature and 63 in both samples. Of these common proteins among the samples, the quantitative analysis of the proteins identified in goat milk showed 22 proteins differentially abundant between the phases of lactation, among which 14 proteins showed more abundant levels in colostrum and 8 proteins showed more abundant levels in the mature. The interaction network between the identified proteins shows four large clusters and seven smaller ones that are divided in relation to biological processes of immunity, energetics, and protein folding. Regarding the proteins identified in colostrum, there was an emphasis on metabolic pathways related to neurodegenerative diseases and energy metabolism. This information may improve the understanding of the functions of proteins in each phase of lactation aiming application in the dairy industry and in the herd.

Keywords: Goat Milk. Nutrition. Phases of lactation.

INTRODUCTION

Goat milk is the most produced in the world, but the least commercialized, behind cow and buffalo milk. In addition to being rich in proteins with great biological value, fat globules are smaller, facilitating their absorption, and it has a large amount of triglycerides that help prevent heart complications (KEBEDE, 2014).

Goat milk has valuable characteristics for human health. It has more minerals than cow's milk, such as calcium, magnesium and copper. Vitamin A is also found in greater amounts and is valuable for human eye and skin health. Goat milk has a lower allergenic potential than bovine milk, as it has a lower protein fraction of alpha-S1 casein (APARNATHI et al., 2017).

For animals, during the gestation of the goat, there is no passage of antibodies from the mother to the animal, so the newborn needs the immunity provided by the food for its survival. Antibodies are acquired from colostrum, which is the milk secreted in the first days postpartum, which is rich in immunoglobulins and ensures that the kid develops in a healthy way.

The intelligent use of goat's milk to generate satisfactory economic effects could be the production of pasteurized milk, UHT, powdered milk, fine cheeses, yogurt, dairy drinks, sweets or other products. In addition to the food sector, goat's milk can be used as a raw material for the cosmetics industry, for the generation of hair and skin care products, due to its high biological potential, originally natural and without toxicity (KAZIMIERSKA; KALINOWSKA-LIS, 2021).

The Saanen breed is originally from Switzerland and is known as the biggest milk producer in the world. In Brazil, the largest herds are in the Northeast region, and the largest milk production is in the Southeast. Due to the large goat milk production and the increase in the use of goat's milk as a raw material for noble purposes, besides the nutritional aspect for newborns, the objective of this work was to characterize and compare the protein profile of colostrum and mature using the LC/MS-MS technique and addressing its biological characteristics in these phases of lactation, seeking information for the creation of formulations for newborn goats that do not have access to colostrum, alongside the application of this knowledge in the dairy industry, to expand the market for goat milk products.

MATERIAL AND METHODS

Ethical aspects

All animals used in this study were kept in strict accordance with the rules of conduct for the use of animals in Teaching, Research and Extension recommended by the Ethics Committee for the Use of Animals (CEUA) of the Universidade Federal de Viçosa - UFV (Protocol No. 59/2019).

Location

The present study was carried out at the Goat Teaching, Research and Extension Unit (UEPE) of the Universidade Federal de Viçosa (UFV), associated with the Department of Animal Science (DZO), and at the Laboratory of Proteomics and Protein Biochemistry of the Department of Biochemistry and Molecular Biology (DBB) at UFV.

Sample collection and preparation

Colostrum samples were collected from 3 Saanen goats, between 2 and 3 days postpartum, in Eppendorf microtubes of 1.5 mL each, totaling 4.5 mL of colostrum milk as a pool. The mature milk samples were collected from 5 Saanen goats, between 2 and 5 weeks postpartum, also in 1.5 mL Eppendorf microtubes, totaling 7.5 mL of mature milk as a pool. The samples were collected on the same day and only one collection was carried out for the execution of the project. After collection, each of the samples was placed in styrofoam with ice and transported to the freezer at -20°C, where they were stored until used.

The colostrum and mature milk pools were defatted by centrifugation at 2,000 x *g* for 15 min at 25 °C. After centrifugation, the fat fraction (cream) was discarded, and the remaining material was recovered and corresponded to skimmed goat's milk.

Determination of soluble protein

The colostrum and mature milk samples were quantified according to the methodology of Bradford (1976). The bovine serum albumin (BSA) was used to make the standard curve in

the quantification of proteins and that the readings were performed in a spectrophotometer (Spectramax type M5), at 595 nm (Supplementary table 1). Then, the total proteins of the samples were separated individually by one-dimensional electrophoresis (SDS-PAGE) (LAEMMLI, 1970). To make a pool for each group (colostrum and mature) 60 micrograms of each individual sample from each group were recovered and added to a new tube.

Four technical replicas of the colostrum pool and four of the mature milk pool were made. Data from one technical replica from colostrum was lost and from one technical replica from mature milk was removed, been considered three technical replica for assays.

Processing of proteins by electrophoresis

Then, the proteins were immobilized and concentrated by the SDS-PAGE Short-Run strategy with concentrations of 4% of the stacking gel and 16% of the separating gel (RESJO et al., 2017; LIEBLER; HAM, 2009). Briefly, the mini gel was made in a Mini-Protean Tetra Cell apparatus (Bio Rad, USA), with 10 channels each, in which 50 µg of total proteins were applied in four technical replicas of the colostrum pool and three of the Maduro group, called Colostrum (CR1, CR2, CR3 and CR4) and Mature (MR1, MR2 and MR3), together with 10µL of positive control (Bovine Serum Albumin – BSA).

The electrophoresis process took place under constant 80 V, which was stopped when the bromophenol blue indicator reached about 10 millimeters from the top of the separating gel.

The mini gel was submerged in a protein fixation solution [10% acetic acid (v/v), 50% ethanol (v/v) and 40% water (v/v)] for 12 hours. Then, the gel was submerged in colloidal Coomassie blue R-250 staining solution for a period of 2 hours (NEUHOFF et al., 1985). After the protein development process, the mini gel was scanned in the ImageScanner III equipment (GE Healthcare, Sweden) in transparency mode and with a resolution of 300 dpi, whose images were stored in the formats (*tif) and (*png). Then, the mini gel remained in storage solution (5% aqueous acetic acid solution), until the enzymatic digestion process.

Protein trypsinization and desalination

The bands from the SDS-PAGE 1D Short-run were excised, sliced and submitted to enzymatic digestion. The enzymatic digestion protocol was performed as previously described by Shevchenko et al. (2006). Briefly, the gel fragments were placed in 1500 µL microtubes

containing 200 µL of 50 % (v/v) acetonitrile in 25 mM ammonium bicarbonate pH 8.0, and washed to remove the dye and SDS. Then, the gels were dehydrated by adding, twice, 200 µL of 100% acetonitrile in each microtube, keeping them at rest for 5 min. The gel fragments were dried for 5 min in a vacuum centrifuge system, model AG-22331 (Eppendorf, Germany).

The proteins present in the gel fragments were reduced in 100 µL of 65 mM DTT in 100 mM ammonium bicarbonate, pH 8.0, at 56 ºC for 30 min. Then, the proteins were alkylated with 100 µL of 200 mM iodoacetamide in 100 mM ammonium bicarbonate, pH 8.0, at room temperature, for 30 min in the dark. Subsequently, the gel fragments were washed, hydrated and dehydrated with ammonium bicarbonate and acetonitrile twice, respectively, and finally, they were dried in a previously referenced vacuum centrifuge system.

In the proteolysis step, the gel fragments were rehydrated in an ice bath, applying 100 µL of porcine pancreas trypsin solution, indicated for use in proteomics, treated with TPCK, reference V5111 (Promega Corporation, USA), in the final concentration of 25ng/µL in activation solution (10% acetonitrile, 40mM ammonium bicarbonate, pH 8.0). After 45 min on ice, 150µL of the activation solution was added to the tubes containing the gel fragments.

The samples were placed in a water bath at 37ºC for 22 h. After proteolysis, the samples were sonicated for 10 min, vortexed for 20 s and the solution was removed to a clean microtube. Next, 150µL of the solution containing 5% (v/v) formic acid in 50% (v/v) acetonitrile were added to the remaining gels. Each tube was vortexed for 20 s, kept at room temperature for 15 min and sonicated for 2 min. The solution was removed and added to the previously reserved solution in the clean microtube. This step was repeated once more, the solution was collected and added to the clean microtube, the same as it was with the previously retrieved solution. The samples containing the peptides were concentrated in a vacuum centrifuge system.

The desalination of the tryptic peptides was processed through C18 reversed-phase micro columns model ZTC18S096 (Millipore, USA), which is called stationary phase. The desalination process was carried out according to the manufacturer's instructions. Briefly, the peptides were resuspended in 10 µL of trifluoroacetic acid – 0.1% TFA solution. The procedure consisted of activating the stationary phase with 100% acetonitrile, equilibrating the stationary phase with 0.1% TFA solution, loading the sample in 0.1% TFA solution, desalting the sample by washing with 0 TFA solution 1% pure, elution of the peptides with 100% acetonitrile and washing of the stationary phase with 100% acetonitrile. The wash solution was recovered and added to the elution solution. The total volume of the recovered peptide solution was taken to

the vacuum centrifuge system to be concentrated. The samples were dried and stored in a freezer at -20 ºC until they were sent for analysis by mass spectrometry.

Sample analysis by LC-MS/MS

The peptides constituting the replicas from the short-run were solubilized in 20 µL of 0.1% aqueous formic acid solution, added 2% (v/v) of LCMS grade acetonitrile), placed in appropriate vials for application in the nano LC-MS/MS system used. After sample preparation, 1 µL of the solution was analyzed by nano LC-MS, using the ultrahigh performance liquid chromatograph – UHPLC NanoAcquity (Waters, USA), containing a trap column nanoAcquity UPLC® 2G-V/MTrap 5 µm Symmetry® C18 180 µm x 20 mm, at a flow rate of 7 µL/min., for 3 minutes. Peptides were separated by a nanoAcquity UPLC® 1.8µm HSS T3 75 µm x 200 mm column, operating at a flow rate of 0.2 µL/min. The mobile phase of the chromatographic process had water acidified with 0.1% formic acid (solvent A) and acetonitrile acidified with 0.1% formic acid (solvent B). Chromatographic separation took place according to the following schedule: 2 % B for 1 minute; gradient from 2% to 30% B for 209 minutes; gradient from 30% to 85% B for 10 minutes; maintenance at 85% B for 5 minutes; gradient from 85% to 2% B for 5 minutes; and maintenance at 2% B for 10 minutes, totaling 240 minutes of chromatographic analysis.

The eluted peptides were automatically injected into a MAXIS 3G mass spectrometer (Bruker Daltonics, Germany), operating in online mode, with a CaptiveSpray ionization source. The peptides were analyzed using an appropriate method for proteomic analysis (IE_captive_nov2019), with a drying gas flow of 3 L/min, ionization source temperature of 150 ºC and transmission voltage of 2 kV. The raw data were converted into a list of masses in the extension *mzXML (Extensible Mark-up Language), using the CompassXport application, version 3.0 (Bruker Daltonics, Germany), which was subjected to identification using the PEAKS software.

Analysis of mass spectrometry data

The lists of masses with extension *mzXML were compared against the database of proteins of the species *Capra hircus* (downloaded on 11/03/2021, with 36,008 entries), deposited at the Uniprot Consortium.

The comparison was performed using the PEAKS application, version 7.0 (Bioinformatics Solutions Inc., Canada) (MA et al., 2003). The parameters used for the research were: enzymatic digestion by trypsin not considering the occurrence of missing cleavage; cysteine carbamidomethylation as a fixed modification and methionine oxidation as a variable modification. Error tolerance for the parental ion of 20 ppm and 0.6 Da for the fragments, considering the analysis of ions with charge +2, +3 and +4. Proteins were identified when they presented at least two unique peptides with FDR (False Discovery Rate) less than one percent. Peptides with a fold-change ≥ 2.0 were defined as differentially abundant. The statistical test used for ANOVA was Pearson´s correlation.

Proteins identified as "Uncharacterized" were analyzed using BLAST software, version 2.4.0 (Altschul et al., 1990). In this analysis, it was possible to identify which proteins deposited in the NCBI non-redundant (nr) protein database showed greater identity with the sequences of the "Uncharacterized" proteins.

Molecular mass, isoelectric point, amino acid composition, and other physicochemical parameters were determined using the ProtParam tool (http://www.expasy.org/tools/protparam.html), available on the Swiss Bioinformatics Resource Portal – Expasy (Gasteiger et al., 2005). All parameters were calculated based on the theoretical amino acid sequence of each identified protein.

Bioinformatics analysis

All proteins identified in goat milk at colostrum and mature stages were analyzed using Gene Ontology (by the PANTHER, http://www.pantherdb.org/) for classification as to biological processes, cellular components and molecular function. They were also analyzed using KEGG pathway analysis (https://www.genome.jp/kegg/pathway.html) to distinguish which metabolic pathways the identified proteins belong to. Finally, using the software STRING 11.0 (SZKLARCZYK et al., 2021) we analyzed the protein interaction networks for all proteins in colostrum and mature milk. The analysis of protein interaction networks was performed by the relationship of evidence between the interaction points presented in the network and the researched database, with reliability greater than 0.9.

RESULTS

Protein identification

Using LC-MS/MS, 228 proteins in total were identified in colostrum and mature milk of goat. The distributions of the proteins among colostrum and mature using the Venn diagram (Figure 1), show the differences and similarities of the samples in presence and absence of some proteins. Among them, 63 proteins were common for both, and 147 were only on colostrum and 18 for mature milk only.

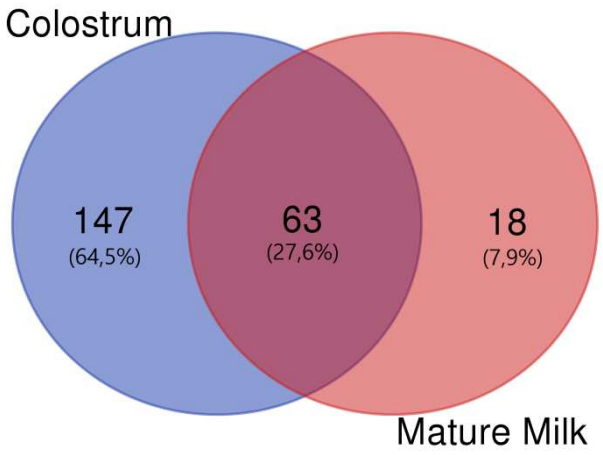


Figure 1: Venn diagram of all proteins identified in goat milk samples, using LC-MS/MS. The distribution is for proteins identified only in colostrum (blue, 147 proteins), only in mature milk (rose, 18 proteins), and in both samples (carmine, 63 proteins). Source: Silva, 2022. Authorial.

Functional classification of the goat milk proteomic profile

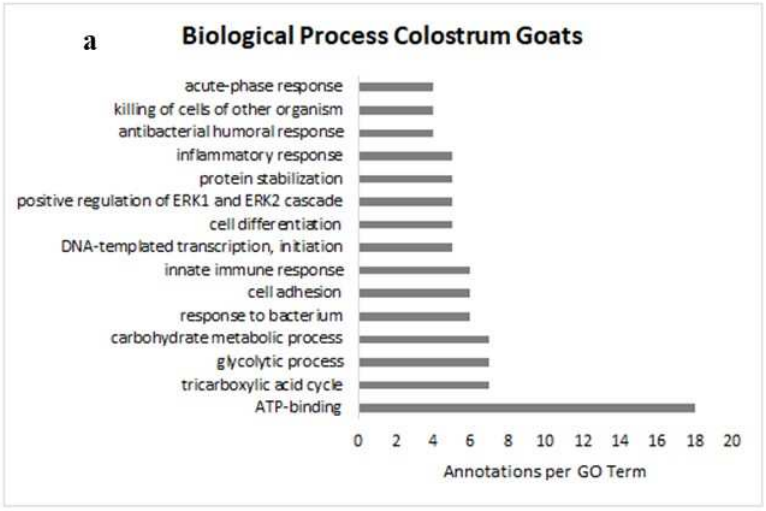
Using Gene Ontology, the 228 identified proteins were classified according to their biological process, molecular function and cellular component (Figure 2 and Figure 3). The samples analyzed in this work - colostrum, mature - show uneven percentages within the distinct classifications.

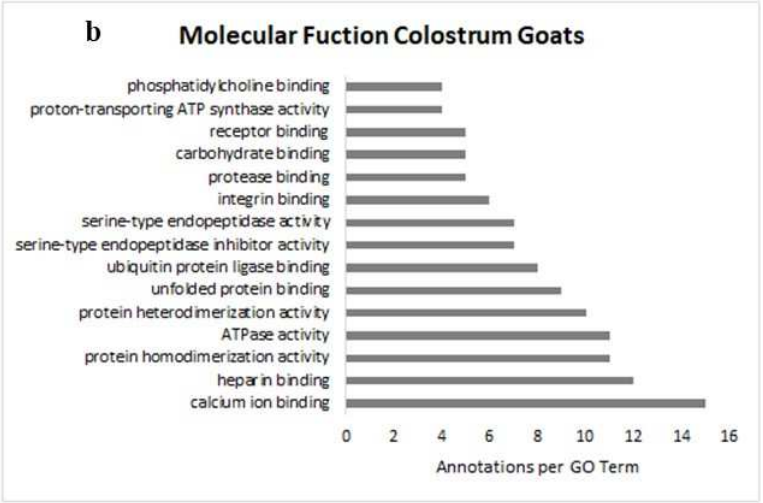
As shown in Figure 2A, the most common biological process in colostrum milk was ATP-binding (18%); tricarboxylic acid cycle, glycolytic process, and carbohydrate metabolic process were ranked second, all with 7%. In the mature milk (Figure 3A) the first biological

process is response to bacterium (7%), followed by cell adhesion, long-chain fatty acid transport, detection of chemical stimulus ISPBT with 6%, 5% and 4%, respectively.

Regarding the molecular function (Figure 2B) in colostrum milk, the main one is calcium ion binding (15%), later comes heparin binding (12%), protein homodimerization activity and ATPase activity, both with 11%. In mature milk (Figure 3B) the first four molecular functions are protein homodimerization activity (6%), calcium ion binding (5%), lipid binding (4%) and protein disulfide isomerase activity (3%).

Figure 2C shows the classification results based on cellular components in colostrum milk and Figure 3C in mature milk. The two first in both samples were extracellular space and extracellular region. In colostrum, these two categories are 35% and 34%, and in mature milk, are 22% and 15%, respectively. This result was already expected because most of the proteins present in milk come from the breast, more specifically in the secretory epithelium of the mammary gland and secreted in the milk receptor within the alveolar lumen.


a
Biological Process Colostrum Goats
acute-phase response
killing of cells of other organism
antibacterial humoral response
inflammatory response
protein stabilization
positive regulation of ERK1 and ERK2 cascade
cell differentiation
DNA-templated transcription, initiation
innate immune response
cell adhesion
response to bacterium
carbohydrate metabolic process
glycolytic process
tricarboxylic acid cycle
ATP-binding
0 2 4 6 8 10 12 14 16 18 20
Annotations per GO Term


b
Molecular Fuction Colostrum Goats
phosphatidylcholine binding
proton-transporting ATP synthase activity
receptor binding
carbohydrate binding
protease binding
integrin binding
serine-type endopeptidase activity
serine-type endopeptidase inhibitor activity
ubiquitin protein ligase binding
unfolded protein binding
protein heterodimerization activity
ATPase activity
protein homodimerization activity
heparin binding
calcium ion binding
0 2 4 6 8 10 12 14 16
Annotations per GO Term

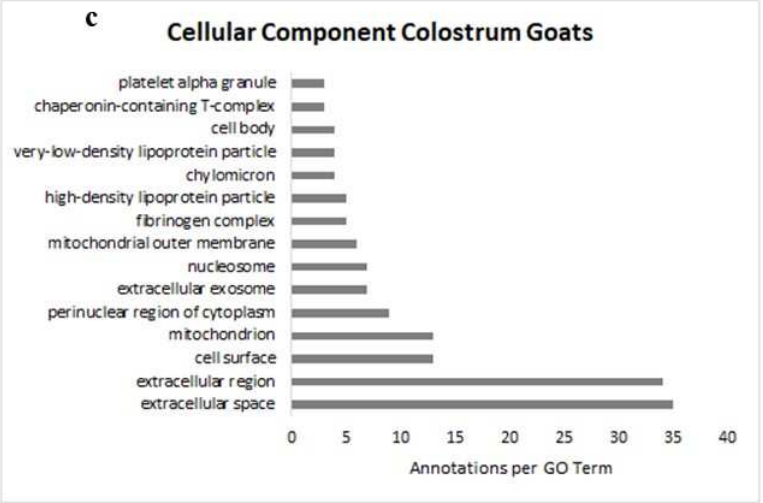
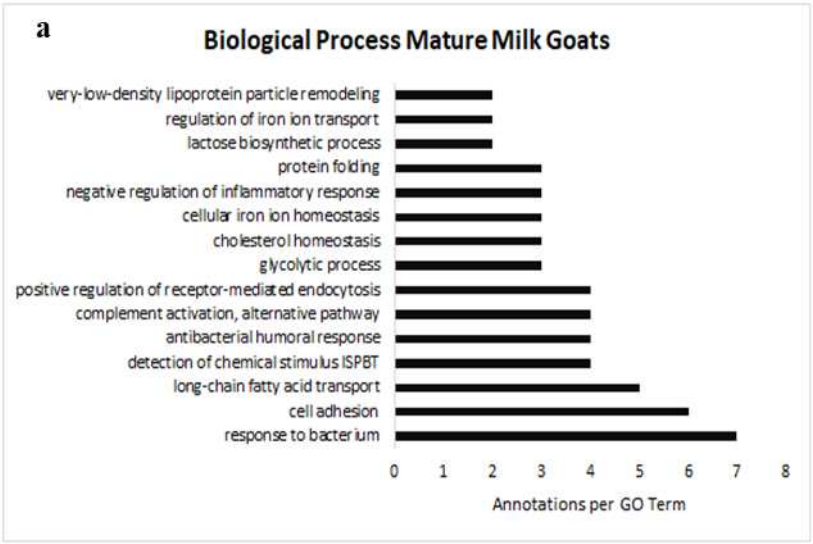
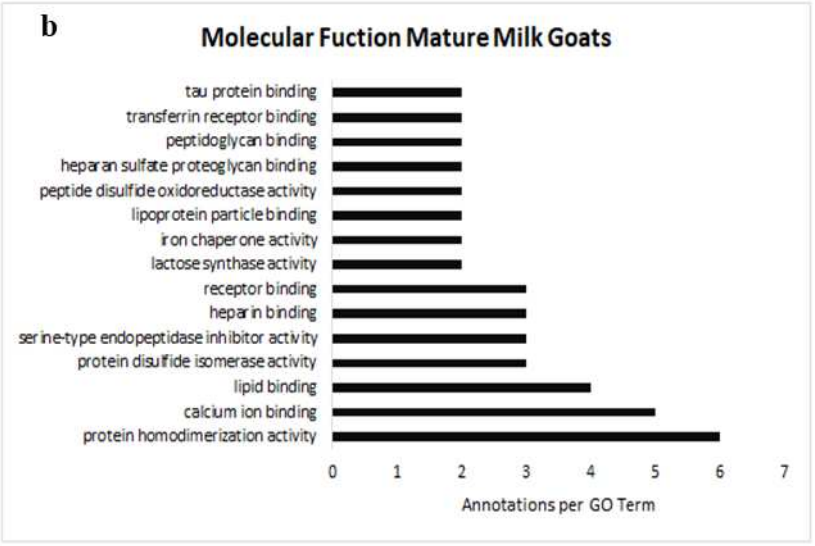

c
Cellular Component Colostrum Goats
platelet alpha granule
chaperonin-containing T-complex
cell body
very-low-density lipoprotein particle
chylomicron
high-density lipoprotein particle
fibrinogen complex
mitochondrial outer membrane
nucleosome
extracellular exosome
perinuclear region of cytoplasm
mitochondrion
cell surface
extracellular region
extracellular space
0 5 10 15 20 25 30 35 40
Annotations per GO Term

Figure 2: Gene Ontology classification of proteins identified in goat colostrum milk on (a) biological process, (b) molecular function, and (c) cellular component. Source: Silva, 2022. Authorial.


a
Biological Process Mature Milk Goats
very-low-density lipoprotein particle remodeling
regulation of iron ion transport
lactose biosynthetic process
protein folding
negative regulation of inflammatory response
cellular iron ion homeostasis
cholesterol homeostasis
glycolytic process
positive regulation of receptor-mediated endocytosis
complement activation, alternative pathway
antibacterial humoral response
detection of chemical stimulus ISPBT
long-chain fatty acid transport
cell adhesion
response to bacterium
0 1 2 3 4 5 6 7 8
Annotations per GO Term


b
Molecular Fuction Mature Milk Goats
tau protein binding
transferrin receptor binding
peptidoglycan binding
heparan sulfate proteoglycan binding
peptide disulfide oxidoreductase activity
lipoprotein particle binding
iron chaperone activity
lactose synthase activity
receptor binding
heparin binding
serine-type endopeptidase inhibitor activity
protein disulfide isomerase activity
lipid binding
calcium ion binding
protein homodimerization activity
0 1 2 3 4 5 6 7
Annotations per GO Term

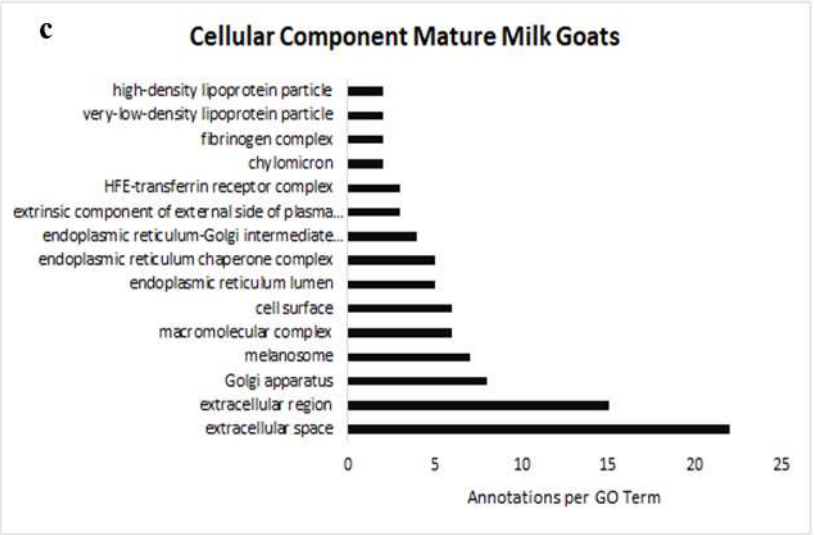

c
Cellular Component Mature Milk Goats
high-density lipoprotein particle
very-low-density lipoprotein particle
fibrinogen complex
chylomicron
HFE-transferrin receptor complex
extrinsic component of external side of plasma...
endoplasmic reticulum-Golgi intermediate...
endoplasmic reticulum chaperone complex
endoplasmic reticulum lumen
cell surface
macromolecular complex
melanosome
Golgi apparatus
extracellular region
extracellular space
0 5 10 15 20 25
Annotations per GO Term

Figure 3: Gene Ontology classification of proteins identified in goat mature milk on (a) biological process, (b) molecular function, and (c) cellular component. Source: Silva, 2022. Authorial.

KEGG pathway analysis

We performed KEGG pathway analysis of all proteins identified in goat milk comparing colostrum (Figure 4) and mature milk (Figure 5). The main pathways in colostrum milk are neutrophil extracellular trap formation at 10% (ranked fourth in mature milk at 7.5%). In mature milk a large number of proteins are involved in complement and coagulation cascades at 10% and in colostrum, at 7.6%.

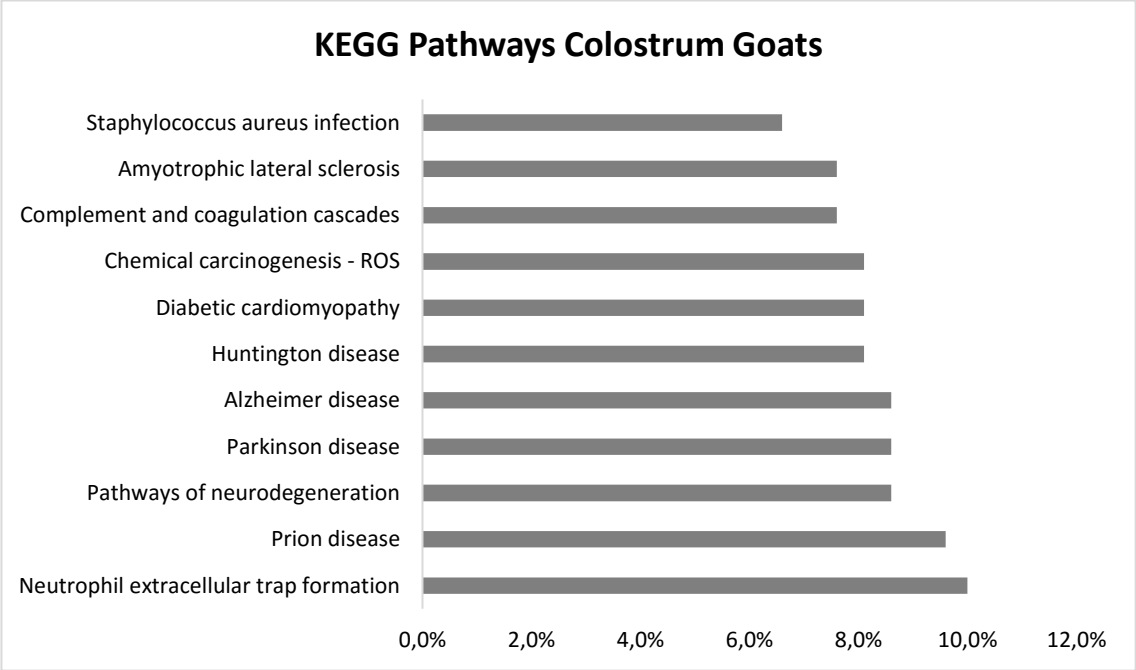


Figure 4: KEGG pathway analysis of all proteins identified in goat colostrum milk. The percentage located in the horizontal part refers to the proteins that are part of that metabolic pathway. The program used to make this analysis was KEGG: Kyoto Encyclopedia of Genes and Genomes version 102.0. Source: Silva, 2022. Authorial.

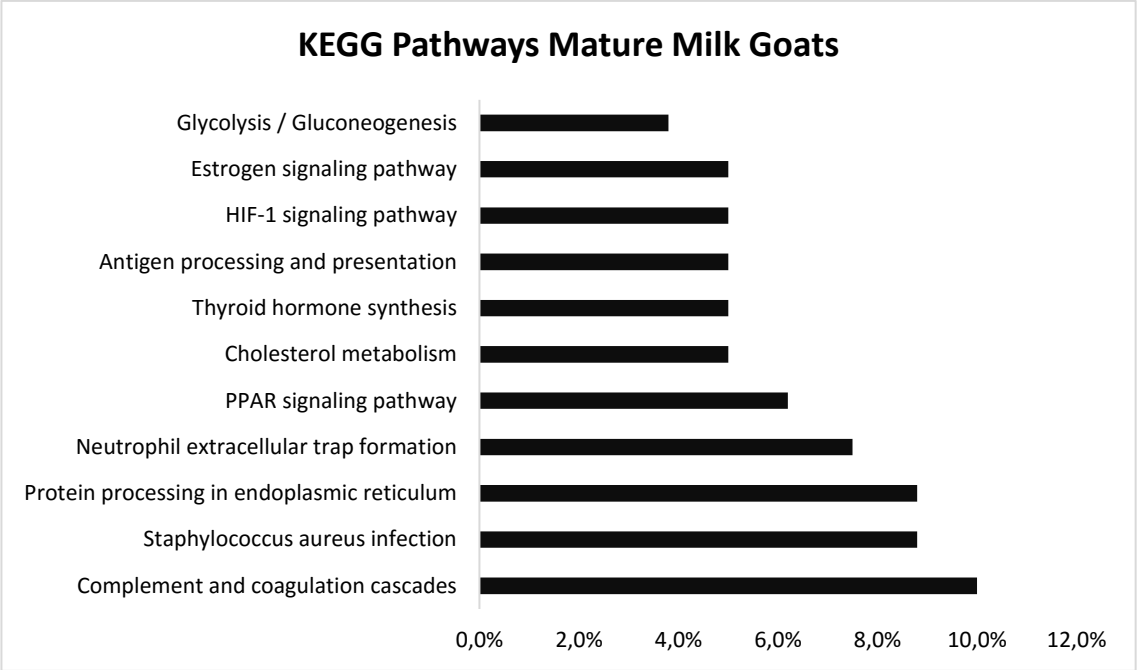


Figure 5: KEGG pathway analysis of all proteins identified in goat mature milk. The percentage located in the horizontal part refers to the proteins that are part of that metabolic pathway. The program used to make this analysis was KEGG: Kyoto Encyclopedia of Genes and Genomes version 102.0. Source: Silva, 2022. Authorial.

Differentially abundant proteins of goat milk

Among the 63 common proteins in goat colostrum and mature milk samples, the quantitative analysis showed 22 proteins differentially abundant between the phases of lactation (Figure 6). Taking into account what was illustrated in the heatmaps, 14 proteins showed more abundant levels in colostrum, which are: vesicle amine transport 1, albumin, transthyretin, Ig-like domain-containing protein, Ig-like domain-containing protein, immunoglobulin mu heavy chain, Gc-globulin, peptidoglycan-recognition protein, fibrinogen alpha chain, fibrinogen beta chain, fibrinogen gamma chain, clusterin, alpha-1-B glycoprotein, and complement C3. In mature milk, eight proteins showed more abundant levels, which are alpha-lactalbumin, immunoglobulin alpha heavy chain, lactadherin, glycosylation-dependent cell adhesion molecule 1, 78 kDa glucose-regulated protein, leucine rich alpha-2-glycoprotein 1, fatty acid binding protein 3, and perilipin (Supplementary figure 2).

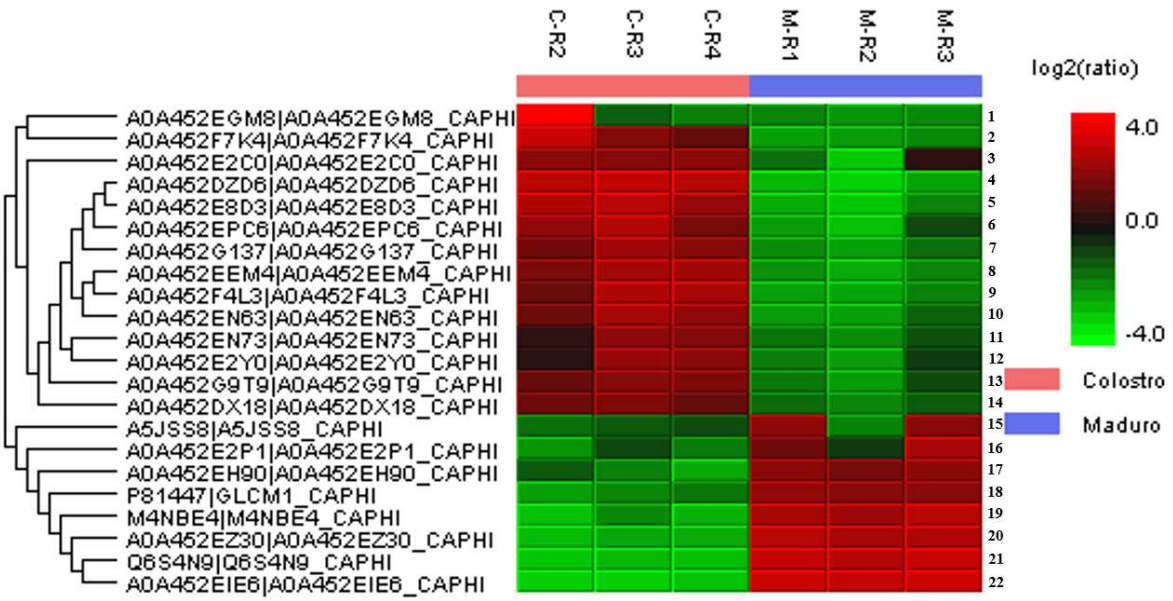


Figure 6: Heatmap for differentially abundant proteins in goat colostrum and mature milk. The red color represents the higher abundant levels, and the green color, the lower levels. The columns show the treatments: C, colostrum (replicates R2-R4); M, mature (replicates (R1-R3). The lines show the proteins. Sequence from one to 14, refers to the most abundant proteins in colostrum, and from 15 to 22, in mature milk. The proteins are: vesicle amine transport 1 (1), albumin (2), transthyretin (3), Ig-like domain-containing protein (4), Ig-like domain-containing protein (5), immunoglobulin μ heavy chain (6), Gc-globulin (7), peptidoglycan-recognition protein (8), fibrinogen α chain (9), fibrinogen β chain (10), fibrinogen γ chain (11), clusterin (12), α-1-B glycoprotein (13), complement C3 (14), α-lactalbumin (15), immunoglobulin α heavy chain (16), lactadherin (17), glycosylation-dependent cell adhesion molecule 1 (18), 78 kDa glucose-regulated protein (19), leucine rich α-2-glycoprotein 1 (20), fatty acid binding protein 3 (21), and perilipin (22). The dendogram on the left side of the image serves to group and assess the similarity levels of the clusters that were formed. The program used to make the heatmap was PEAKS version 7 and the statistical test used for ANOVA was Pearson´s correlation. Source: Silva, 2022. Authorial.

Protein interaction networks

The interaction networks involving the proteins identified in colostrum and mature milk (Figure 7) defined four major clusters (f, g, j, and k) and seven minor ones (a, b, c, d, e, h, and i).

The clusters a, b, c, d, e, and f (Figure 7) are more related in the biologic process of immunity, as humoral immune response, complement activation and immune response. In these clades, they have 17 proteins identified only in colostrum milk and none of them identified only in mature milk. Some of the related proteins are myeloperoxidase, complement C1s, adipsin, among others. Cluster f has proteins that act in the transport and fluidization of molecules to prevent their precipitation and in the coagulation process. As an example, α-1-acid glycoprotein which functions as a transport protein in the bloodstream. And the fibrinogen gamma chain which is a clotting protein, its concentration increases rapidly in response to inflammatory processes.

Already the clusters g, h, i, and j (Figure 7) have a higher energetic character. In these clades, they have 29 proteins identified only in colostrum milk and two identified only in mature milk. More specifically, the g cluster is related to the proteins that participate in the glycolytic pathway. For example, hexokinase (protein identified only in colostrum), the first enzyme to act on glycolysis and transform it into glucose-6-phosphate, the main substrate for metabolic pathways. The pyruvate kinase participates in the tenth step of glycolysis, by transferring phosphate from phosphoenolpyruvate (PEP) to ADP, forming a molecule of pyruvate and another of ATP. Cluster j represents the proteins involved with the supply and functioning of the Krebs Cycle and the electron transport chain. The protein oxoglutarate dehydrogenase is what controls the citric acid cycle. And NADH dehydrogenase is the first enzyme in the electron transport chain, it catalyzes the oxidation of NADH and reduces coenzyme Q.

Lastly, the cluster k (Figure 7) reports proteins involved with the biologic process of protein folding, five of which were identified only in colostrum and five were identified only in mature milk. Some of the proteins present are CCT-alpha, HATPase_c domain-containing protein e protein disulfide-isomerase.

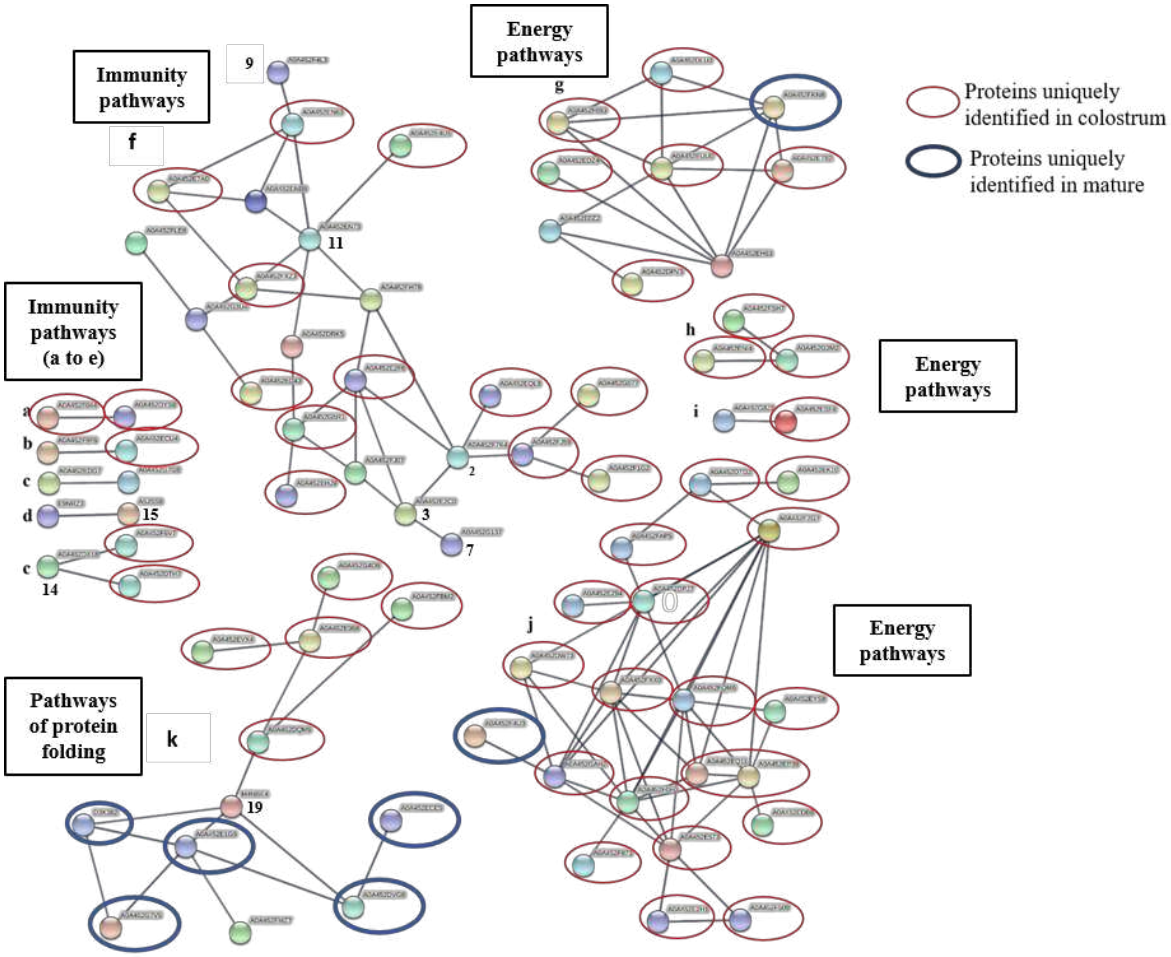


Figure 7: Interaction networks of proteins identified in goat colostrum and mature milk. The proteins circled in red were identified only in colostrum, those circled in blue were identified only in the mature milk, and those not labeled were the proteins identified in both samples. Clusters a, b, c, d, e, and f are related with immunity processes; clusters g, h, i, and j report proteins involved in the energetic metabolism; and cluster k reports proteins involved in protein folding process. The numbers 2, 3, 7, 9, 11, 14, 15, and 19 refer to the proteins reported in Figure 6. The networks were obtained through the software STRING 11.0. Source: Silva, 2022. Authorial.

DISCUSSION

In this work, nano LC-MS/MS analysis was used to characterize the differential protein profile on the colostrum and mature phases in Saanen goat milk. The differentially abundant proteins in each sample were evaluated about their biological functions in goat milk.

For colostrum samples, considering the classification of proteins according to the involved biological process, calcium ion binding ranks first in molecular function. This happens because casein phosphopeptides act as transporters and non-transporters of minerals, forming complexes with calcium ions and more mineral ions, causing their absorption to be greater. In turn, calcium is important for bone health, growth and metabolism (PARK, 2015).

For the mature sample, regarding classification of proteins according to the involved biological process, the third place is occupied by lipid binding. Lipid is a fundamental molecule for several biological processes. It functions as substrates for energy production, in specific signaling processes and as components of biological membranes. Because lipids are hydrophobic, they are not easily transported by aqueous environments, such as soluble cytoplasm and blood plasma. However, there are proteins that are able to bind to lipids and cause the transport to be carried out, these proteins are called 'lipid binding proteins' (GLATZ, 2015).

Colostrum samples show molecular functions involved with the tricarboxylic acid cycle, glycolytic process, and carbohydrate metabolic process. These processes occur in the mitochondria and/or cytoplasm, which is in accordance with the cellular components involved, which are mitochondria and the perinuclear region of the cytoplasm.

KEGG pathway analyses of all proteins identified in goat colostrum milk allow us to notice that, for both milk samples, the pathway is firstly related to immunity. Colostrum samples showed a high percentage of proteins involved in neutrophil extracellular trap formation. The molecule, neutrophil extracellular trap (NET), has an important role in the defense system against bacteria, fungi, viroses, and parasites, because NET trap neutralize and kill the microorganisms and prevent bacterial and fungal dissemination (PAPAYANNOPOULOS, 2017). In mature milk samples, 10% of the proteins participate in complement and coagulation cascades, which are highly important for innate immune systems, as they participate in defense against pathogens (YANG et al., 2017).

Furthermore, considering KEGG pathway analysis, more than 50% of proteins in colostrum samples are related with neurodegenerative disease (Prion, Parkinson, Alzheimer and Huntington disease, Diabetic cardiomyopathy, and Amyotrophic lateral sclerosis) and pathways neurodegenerative. This accords with DEONI et al. (2018) that carried out research with human milk and reveal that breast milk provides short- and long-chain polyunsaturated fatty acid, biofactors, hormones, phospholipids, neurotrophic factors, and proteins that are important for the elaboration of myelin, as a fundamental component of the myelin sheath and/or energy

sources. Deficiency of these components during childhood can significantly change the morphology, content and composition of myelin, capable of disrupting normal brain function and cognitive outcomes. This important function is not detected in the mature milk, as the involvement of proteins with these diseases were not ranked.

Differentially abundant proteins in goat colostrum milk

The albumin is transported in the blood to the mammary gland by passive transfer. The barrier blood-milk only stays permeable during the colostrogenesis and establishment of lactation, so it's possible for the blood components to pass to milk and vice versa. But during the lactation and with the presence of hormones, the barrier is closed and it isn't allowing for transfer (WALL et al., 2015). Therefore, albumin indicates that the blood-milk barrier is permeable. According to the studies of Kebede (2014) and Hernández-Ledesma, Ramos e Gómez-Ruiz (2011), the development and growth of breast cancer cells are inhibited by albumin. So, albumin plays an important role in the immune system.

The peptidoglycan-recognition protein develops several functionalities against microbial and tumor. In Gram-positive bacteria, the protein acts by recognizing the muramyl pentapeptide or tetrapeptide. The peptidoglycan-recognition protein also binds to Gram-negative bacteria because it uses binding sites outside of the peptidoglycan-binding groove (WOLF; UNDERHILL, 2018).

The main function of transthyretin is the transport of retinol (vitamin A) and of the thyroid hormone (TH) thyroxine (MAGALHÃES; EIRA; LIZ, 2021). Both molecules have important functions in the body, as vitamin A is an important micronutrient for the health, related with the functioning of the visual system, development and growth, immune, and reproductive function, maintenance of epithelial integrity, red cell production and others (SOARES et al., 2019), and the thyroid hormone (TH) thyroxine are fundamental for development and growth in most vertebrates (MENDOZA; HOLLENBERG, 2017). The interaction networks of proteins obtained in this work show that transthyretin is involved with small molecule metabolic processes.

The immunoglobulin mu heavy chain defines the classes of immunoglobulin, IgM. IgM is the main product in the primary immune response to infectious agents or antigens, but it is also relevant for immunological tolerance and immune regulation (SHEIKH et al., 2022) (LIU et al., 2019).

The Gc-globulin is the main carrier protein of vitamin D and her metabolites, also performs others relevant biological functions, as actin scavenging to fatty acid transport and macrophage activation, macrophage chemotaxis and can play a role in bone density (SPEECKAERT et al., 2006).

The complement C3 is a fundamental constituent of the innate immune system, which combines with other proteins of complement, making a major host mechanism that detects and eliminates potential pathogens. This protein relates with at least 25 different soluble membrane bound proteins and activates the three complement pathways: lectin, classical and alternative pathway (DELANGHE; SPEECKAERT; SPEECKAERT, 2014). According to the protein network, this protein is involved with 11 biologic processes related to immunity like humoral immune response, complement activation and immune response. Only one is related to metabolic processes, that of small molecules.

The fibrinogen alpha, beta and gamma chain are important for inflammation, hemostasis, wound healing and angiogenesis. This is because it forms an insoluble gel when converted to fibrin by action of serine proteolytic enzyme thrombin. This stable and proteolysis resistant mechanism is essential to prevent blood loss and ensure wound healing (WEISEL, 2005). The interaction networks of proteins exhibit that the fibrinogen alpha and beta are involved in seven immunological processes, including immune response, adaptive immune response, and response to other organisms.

The clusterin is an ATP-independent molecular chaperone and stress-activated. It plays an essential role in protein homeostasis/proteostasis, inhibition of cell death pathways, and modulation of pro-survival signaling and transcriptional networks (WILSON; ZOUBEIDI, 2016).

Differentially abundant proteins in goat mature milk

The alpha-lactalbumin plays an essential role for the time of milk production. It is produced in the epithelial cells of the mammary gland, and with the beta-1,4-galactosyltransferase they form lactose synthase, converting glucose and galactose into lactose. The formation of lactose creates an osmotic force to attract water into the mammary gland and lead the total volume of milk produced (LAYMAN; LÖNNERDAL; FERNSTROM, 2018). The alpha-lactalbumin is involved with carbohydrate metabolic processes, as shown in interaction networks of proteins.

The immunoglobulin alpha heavy chain defines the classes of immunoglobulin, IgA, which is the main serum immunoglobulin and is located in the secretions, like surfaces of the gastrointestinal, respiratory, and genitourinary tracts. So, IgA acts as an essential first line of defense (WOOF; KEN, 2006). The molecule is fundamental to protect against toxins, viruses, and bacteria across direct neutralization or prevention of binding to the mucosal surface. IgA is also important for preventing bacterial or viral infections, pathogenesis, or both when it is in the intracellular region (SCHROEDER; CAVACINI, 2010).

Lactadherin, the most abundant protein in mature milk, is one of the main proteins present in the milk fat globule membrane (MFGM). This protein is related to antiviral and microbial effects, and is important to immune defense similarly to the immune system molecules. The lactadherin also belongs to secreted extracellular matrix proteins family and it is considered as a multifunctional glycoprotein related in regulation of many physiological and biological processes, like angiogenesis, atherosclerosis, haemostasis, phagocytosis, and tissue remodeling (SABHA et al., 2018).

The glycosylation dependent cell adhesion molecule 1 (GlyCAM-1) is a sulfated glycoprotein mucin-like, it has some functions as epithelial protection, lubrication and cellular transport and secretion of milk (MUNIZ et al., 2006). Glycan chains increase the diversity and functional roles of proteins. For example, pathogenic bacteria recognize and adhere to patterns of glycans present in the intestinal mucosa. The glycans present in the glycoproteins of ingested milk can act as a trap, preventing bacterial adhesion (VALK-WEEBER et al., 2021).

The 78 kDa glucose-regulated protein (GRP78) is an endoplasmic reticulum chaperone and a member of the HSP70 protein family. According to the LIU et al. (2019), GRP78 is also a positive regulator of milk biosynthesis and the proliferation of bovine primary mammary epithelial cells (BMECs). It responds to amino acids and hormones, as extracellular stimuli, and stimulates mTOR signaling, leading to milk biosynthesis and increased cell proliferation. The mTOR is an essential kinase that regulates protein and fat synthesis, cell cycle progression such as cell functions, cell proliferation. The mTOR controls umpteen downstream targets, including S6K1, 4EBP1, SREBP-1cand cyclin D1, to induce milk biosynthesis and cell proliferation.

The leucine rich alpha-2-glycoprotein 1 domain-containing protein, according to Zou et al. (2022), is responsible for activities of the nervous system, such as synapse formation, synapse growth, the development of nerve processes, neurotransmitter transfer and release, and cell adhesion molecules or ligand-binding proteins.

The fatty acid binding protein (FABP) is responsible for fatty acid transport and lipid

metabolism. Recently, it was discovered that FABP can also play a role in regulation of cell proliferation and differentiation, at least, in the mammary gland (SPITSBERG; GOREWIT, 2002).

The perilipin plays an important function in milk lipid synthesis and secretion (PAVLOVA et al., 2020). For the growth and development of the newborn animal is essential the lipid, because it gives calories, essential fatty acids, and bioactive lipids. Because of this, the mammary glands show a great ability to synthesize and secrete a wide number of lipids during lactation (MCMANAMAN, 2009).

Interaction network of proteins

The interaction network of proteins obtained in this work shows that the proteins identified in colostrum are in greater numbers in the three groups of biological processes, which are immunity, energetic and protein folding. In colostrum, the biological processes related to energy stand out, due to the large energy expenditure for milk production in the mammary glands. Then, the immunity processes, as the puppy is born with its weak immune system, and it is necessary to strengthen it through breast milk.

Ultimately, seven proteins of colostrum milk are involved in protein folding. The protein folding process generates biological activity, in addition to being associated with several biological processes, including the traffic of molecules to specific cellular sites and the regulation of cell growth and differentiation. So proteins that do not fold correctly, or do not remain correctly folded, are the source of a wide variety of pathological conditions (DOBSON, 2003). According to the information presented, the complexity of colostrum milk is evident and how it is a complete food for the newborn.

The proteins identified in mature milk are also involved in the three biological processes mentioned above. In turn, it has the same number of proteins identified in the processes related to immunity and defense, and a smaller number in the protein folding process.

Proteins with defense functions have decreased in relation to colostrum, but they continue to be provided, because the puppy's immune system is more competent. Proteins related to energy also decreased, as the animal already has other sources of food.

CONCLUSION

The characterization of the proteomic profile of Saanen goat milk in the colostrum and mature lactation phases evidenced that differentially abundant proteins are present among groups, showing distinct biological functions. These proteins exert specific functions for each growth stage of the newborn goat.

The great involvement of goat colostrum proteins with the metabolism of neurodegenerative diseases may indicate the presence of proteins participating in the process of formation of the myelin sheath; this must guarantee the normal functioning of the animal's brain.

Another highlight is the proteins involved in protein folding, which were found in both stages of breastfeeding and that are different in colostrum and mature milk samples. This finding indicates variability of proteins for this function.

This work also evidenced the wide involvement of goat colostrum proteins with the metabolic pathways of energy synthesis. The energy metabolism and the protective metabolism against neurodegenerative diseases were more prominent than the immunity processes and defense pathways in Saanen goat colostrum.

Along with molecules associated with defense, the supply of biomolecules that offer energy and protection of the neural system showed to be essential to ensure the healthy development of newborn kids, also visualizing the possibility of offering goat products for newborn children, added of components that favor neural, immune and physical health.

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Supplementary table 1. Determination of soluble protein. Source: Silva, 2022. Authorial.

Samples	Reading Empty Plate (nm)	Reading Samples (nm)	Reading Samples - Reading Empty Plate	Average	Concentration (ug)	Concentration (ug/uL)
Mature Milk 1	0,102	1,728	1,626	1,609	56,844	11,369
	0,049	1,661	1,612			
	0,037	1,627	1,59			
Mature Milk 2	0,045	1,585	1,54	1,547	51,923	10,385
	0,055	1,609	1,554			
	0,047	1,595	1,548			
Mature Milk 3	0,047	1,571	1,524	1,521	49,860	9,972
	0,045	1,579	1,534			
	0,047	1,553	1,506			
Mature Milk 4	0,044	1,57	1,526	1,487	47,161	9,432
	0,114	1,535	1,421			
	0,044	1,559	1,515			
Mature Milk 5	0,044	1,535	1,491	1,460	45,019	9,004
	0,044	1,538	1,494			
	0,051	1,447	1,396			
Colostrum Milk 1	0,046	1,619	1,573	1,545	51,738	10,348
	0,051	1,598	1,547			
	0,047	1,562	1,515			
Colostrum Milk 2	0,046	1,626	1,58	1,542	51,526	10,305
	0,046	1,566	1,52			
	0,047	1,574	1,527			
Colostrum Milk 3	0,045	1,554	1,509	1,539	51,288	10,258
	0,241	1,703	1,462			
	0,045	1,692	1,647			

Supplementary table 2. Identified proteins in the Saanen goat milk using nano LC MS/MS. Source: Silva, 2022. Authorial.

ID	Protein name	Protein score (-10lgP)	MW (kDa)[a]	pI [a]	Peptide sequence	Coverage (%)	Identified
A0A2U8URJ7	Beta-casein (Fragment)	140	15079.78	6.45	YPDEPFTESQSLTLTDVEK	51.5	Both samples
					VLPVPQK		
					DMPIQA		
					LHLPLPLVQSWMHQPPQPLCPTVMFPPQSVLSLSQPK		
A0A452DPW0	Chitinase-3-like protein	163,09	42954.83	8.75	TDVGAPISGPGIPGR	23.2	Colostrum
					TLLSVGGWNFGPER		
					GYDIAQISR		
					THGFDGLDLAWLYPGR		
					GNQWVAYDDQESVK		
					SVPPFLR		
					DVLAEV		
					SFTLASSK		
A0A452DRK5	Hemopexin	209	51945.84	7.27	FPGIPFPLDAAVECHR	33.1	Both samples
					DAPSPVDAAFR		
					EFGSPQGVSLHSVDAAFTCPGFSR		
					GEFVWK		
					LWWLDLK		
					VDGALCTEK		
					TWFWDFSTSTIK		
					FDPIEGK		
					YDHNSVLLIK		

					YYCFR		
					MNSLLGCAPSQHS	15	Colostrum
					APDHGHGVEGGNVAKPDADVTER		
					DYFMPCPNR		
A0A452DRX9	Sperm acrosome associated 1	131	30924.73	4.42	AFECETLDNNEIVASIR		
					ASTTEIQSELSSMR		
					LACVHTSPENR		
A0A452DTC0	Histone H2A	80	14121.47	11.05	AGLQFPVGR	21.5	Colostrum
					VTIAQGGVLPNIQAVLLPK		
A0A452DTH7	Complement factor I	160	68558.95	7.73	MGDFPWQIAIK	9.1	Colostrum
					VANYFDWISHHVGR		
					GLETSLAECTFTK		
					SFECVRPEAK		
					VFCQPWQR		
A0A452DTM3	Biglycan	77	41957.34	7.18	IQAIELEDLLR	5.36	Colostrum
					VPAGLPDLK		
A0A452DX18	Complement C3	318	187307.46	6.07	TGIPIVTSPYQIHFTK	42153	Both samples
					VHQYFNVGLIQPGAVK		
					VELLYNPAFCSLATAK		
					ILLQGTPVAEMAEDAIDGER		
					DYAGVFTDAGLTFK		
					VELKPGETLNVNFHLR		
					EYVLPSFEVQLEPEEK		
					ADIGCTPGSGR		

TIYTPGSSVLYR
SSVAVPYVIVPLK
HQQNIMIPAR
VGLHEVEVK
FYYVDDPDGLK
SDLDDEIIPEEDIISR
VYSYYNLDETCIR
TMQALPYNTQGNSNNYLHLSVPR
VGQYSGDLR
ISLTHSLTR
LYNVEATSYALLALLAR
ILWESASLLR
HIPVVTQGSNVQSLTQDDGVAK
ACEPGVDYVYK
LLSTGVDR
LLPVGQTVFISIETPDGVLVK
LPYSVVR
VFALSANLIAIDSR
AILYNYR
VVPEGIR
GYTQQLAFR
LVAYYTLINANGQR
AAVYNHFISDGVK
EGALELAR
NEQVEIR
IWDVVEK

					GSMILDICTK		
					DPLTITVR		
					AHYEDSPQQVFSTEFEVK		
					TLNPEYLGQDGVQR		
					SSAYAAFQNRPPSTWLTAYVVK		
					EPGQDLVVLPLTITSDFIPSFR		
					AFLDCCEYIAR		
					DICEAQVNSLGR		
					ADALVGK		
					DLCETVK		
					EVVADSVWVDVK		
					GVFVLNK		
					WLILEK		
					RPYTVAIAAYALALLGK		
					VPINDGNGEAILK		
					VSHTQEDCLSFK		
					NTLIIYLDK		
					FFKPAMPFDLMVYVTNPDGSPAR		
					WLNEQR		
					AGNFLENHYR		
					DSCMGTLVVK		
					ELNLDVSIHLPSR		
A0A452F7S5	Beta-2-microglobulin	136	13699.54	6.17	IPEVQVYSR	24.2	Both samples
					SEQSDLSFSK		
					VNHVTLTQPK		

A0A452F854	SERPIN domain-containing protein	169	46482.22	5.64	IFTDAADLSGVTGTR	70.6	Both samples
					IHELYLPK		
					GSTLTEILEGLK		
					DTQSIIFLGK		
					SNYELNDILFQLGIK		
					SLINDYVK		
					LIPETLTR		
					IEELFK		
					HTEQAEFHVSK		
					AHVDDHTLASSNTDFAFSLYK		
					SNYELNDILFQLGIEK		
					FDRPFLIAVVLK		
					FSISSDYNLEDTLSELSIQK		
					FNRPFLIAAILK		
					LIHTLYLPK		
					WQIPFDPK		
					IVDLFMDLDPLTK		
					LSPETLTR		
					DAEAFYASEVLPINFK		
					GPTVAEILEGLK		
					FSISSDYQLK		
					TYDSEFLVSK		
					DILSHLGMK		
					DTQSIIFLAK		

					LINEYVK		
					MQDLEAK		
					SAEAVLGEALTDFSLR		
					DTFAEASQR		
					LILLNAVALSAK		
					YPVASFTDPTLK		
					LLDSLPEDTR		
					FHPTHLTMPR		
					LYHDFSVLK		
					AFTSEGFTSFSQIFHSSDLAIR		
					FPVFMGR		
A0A452F9F6	SERPIN domain-containing protein	158	51630.09	5.59	SAEAVLGEALTDFSLR	23	Both samples
					DTFAEASQR		
					LILLNAVALSAK		
					YPVASFTDPTLK		
					LLDSLPEDTR		
					FHPTHLTMPR		
					LYHDFSVLK		
					AFTSEGFTSFSQIFHSSDLAIR		
					FPVFMGR		
A0A452FAW4	Lipopolysaccharide-binding protein	96	53468.01	6.81	IQLYDPILQIHK	4.8	Colostrum
					VTLPNFDGDVR		

A0A452FG03	Annexin	63	38897.58	6.37	GLGTDEDTLNEILASR	15	Colostrum
					CLTVIVK		
					SEELAVNDDLADSDAR		
					GVDEATIIEILTK		
A0A452FH78	Alpha-2-HS-glycoprotein	147	38184.54	5.25	TPIVGQPSVPGGPVHLCPGR	17.3	Both samples
					HTLNQIDSVK		
					QDGQFSVLFTK		
					HTFSGVASVESASGEAFHVGK		
A0A452FHJ3	Actin-depolymerizing factor	125	85577.36	5.98	TGALELLR	9.1	Both samples
					AQPVQVAEGSEPDSFWEALGGK		
					AGALNSNDAFVLK		
					HVVPNEVVVQR		
					QTQVSVLPEGGETPLFK		
A0A452FHZ0	Lipocln_cytosolic_FA-bd_dom domain-containing protein	154	23017.36	9.44	SLGLTDDHIIFPVPIDR	37.5	Both samples
					IPLQPNFQADQFQGK		
					WYAVGVAGNAIK		
					TFVPSSRPGQFTLGNIR		
					ITLYGR		
					ENFITFAK		

A0A452FI14	Apolipoprotein A1	191	30322.39	5.71	LLDNWDTLASTLSK	47.5	Both samples
					VAPLGEEFR		
					VSILAAIDEASK		
					EGGGSLAEYHAK		
					LSPLAQELR		
					QGLMPVLESLK		
					EQLGPVTQEFWDNLEK		
					AKPALEDLR		
					QQLAPYSDDLR		
					DYVAQFEASALGK		
					VQPYLDEFQK		
A0A452FJ07	SERPIN domain-containing protein	182,45	48709.97	5.71	IAPNLANFAFSIYHK	24.9	Both samples
					NLYHSEAFSINFR		
					TVLGELGINK		
					SPLFVGK		
					AALTIDEK		
					YASSANLHLPK		
					LSISETYDLK		
					INDYVEK		
					DFHVNEQTTVK		
					VFSNGADLSGITEEQPLK		
A0A452FJ59	Fibronectin	116	246036.53	5.48	LGVRPSQGGEAPR	3084.5	Colostrum
					VPGTSASATLTGLTR		

					FTQVTPTSLTAQWTAPNVQLTGYR		
					VTWAPPSSIELTNLLVR		
A0A452FJF9	Beta-1 metal-binding globulin	244	76952.01	6.63	TYDSYLGDDYVR	41.1	Both samples
					DKPDNFQLFQSPHGK		
					YYGYTGAFR		
					FDEYFSAGCAPGSQR		
					TAGWNIPMGLLYSK		
					ASDADLNWNNLK		
					ELPDPQESIQR		
					ATCVEQILK		
					DNPQTHYYAVAVAK		
					GDFELLCK		
					AAASFFSASCVPCADQSSFPK		
					SAGWNIPMGR		
					GDVAFVK		
					DSADGFLK		
					IFENGPFVSCVK		
					AVTDCTSNFCLFQSTSK		
					LCQLCAGK		
					CLAEGSGDVAFVK		
					DLLFK		
					SVDDYHECHLAK		
					ECVPNSNER		
					WCAIGHQER		
					HQTVTQNTNGNNDEAWAK		

					DYELLCGDNTR		
					ISCTIANR		
A0A452FKE1	L-serine ammonia-lyase	130	34298.01	8.39	ALGVTTVTAQAMK	25.8	Colostrum
					VAGTTVYLK		
					EHPIFSEVISDQEAVAALEK		
					VVGETLDEAIR		
					LDSAQPSGSFK		
					LGIPATIVVPNTTPALTIQR		
A0A452FLE8	Apolipoprotein E	170	37238.40	6.00	LRPLVEQGQSR	31.9	Both samples
					SEVQAMLGQSTEELR		
					LAVYQAGASEGAER		
					SWFEPLVGDMQR		
					LQAEAFQAR		
					QWAGLVEK		
					LWDYLR		
					GQDSQPWEQVLGR		
					LEEVGVR		
					LGSDMEDLR		
B6D982	Serum amyloid A protein	130	14675.52	9.41	ETIQGITDPLLK	23,7	Colostrum
					ADQFANEWGR		
					GPGGAWAAK		

A3EY52	Butyrophilin subfamily 1 member A1	270	59265.83	5.11	TPDEEGLFTVR	42.8	Both samples
					VSPAVFVSR		
					TPLPLAGPPR		
					VAALGSDPHINMK		
					QDENYEEAIVHLK		
					KPLTICPITDGLEGVMVVADAK		
					EQEGEEMAEYR		
					VSLVEDHIAEGSVAVR		
					THEGEEFPSMSESR		
					EVEISIPASFFPR		
					EIPLSPMGEDSASGDIETLHSK		
					TDWAIGVCR		
					NLLLGQEK		
					ATLHAVDVTLDPDTAHPHLFLYEDSK		
					ASFSGPLRPFFCLWSCGK		
					FDSWPCVMGR		
A0A452FP43	Gal_mutarotas_2 domain-containing protein	75	109521.96	5.77	GLLNLEHQR	4,9	Colostrum
					YHGPQTLYLPVTLSSIPVFQR		
					MLDYLQGSGETPQTDVR		
A0A452FQC0	Cathelicidin-1	159,67	17606.09	6.58	AVDQLNEQSSEPNIYR	29	Both samples
					ICQFVLIR		
					QPWAPPQAAR		

					CEGTVTLDQVR		
A0A452FTD0	Ig-like domain-containing protein	153	18762.81	6.71	DSERPSGILDR	26,4	Colostrum
					TATLTISGAR		
					YTAWYQQKPGQAPVK		
					ITCQGDLLDEK		
A0A452G0P6	IF rod domain-containing protein	144	58036.33	5.01	GSLGGGFSSGGFSGGSFSR	20.6	Both samples
					LENEIQTYR		
					QSLEASLAETEGR		
					VLDELTLTK		
					LASYLDK		
					DAEAWFNEK		
					LAADDFR		
					QSVEADINGLR		
					AGLESTLAETECR		
					ILAATIDNNR		
					IILEIDNAR		
A0A452G137	Gc-globulin	185	52366.90	5.41	VPTAHLEDVLPLAEDITTILSK	39.1	Both samples
					ICSQYAAYGK		
					LPDVQLPTNK		
					ELSSFIQK		
					GNTNVLDQYIFELSR		

					HQPQEFPTYVEPTNDEICEAFR		
					ILEPTLK		
					SLDECCHSESSAACLNEK		
					GQELCADYSENTFTEYK		
					ELPEYTVK		
					SYLSMVGSCCTSPNPTACFLK		
					SCESNSPFPVHPGTPECCTHEGLEK		
A0A452G1U2	Glycoprotein IIIb	134	52830.28	8.62	VAIIDTYK	16.7	Both samples
					EVVLEDGTIAFK		
					LQINILVKPAR		
					FVLPALAFASPLQNPDNHCFCTEK		
					TLQFFSSDICR		
					SIYAVFGAEMNLK		
A0A452G3U4	Lipoprotein lipase	123.85	53700.24	8.80	LSPDDADFVDVLHTFTR	11.5	Both samples
					LVAALYK		
					GLGDVDQLVK		
					EPDSNVIVVDWLSR		
					SPVIFVK		
A0A452G7G8	Joining chain of multimeric IgA and IgM	136.77	18033.59	5.90	IIPSAEDPSQDIVER	25.3	Both samples
					IVLVDNK		
					IIVPLNSR		
					FVYHLSDLCK		
A0A452G8J3	Actin gamma 1	238	41792.84	5.31	TTGIVMDSGDGVTHTVPIYEGYALPHAILR	80.8	Both samples

					SYELPDGQVITIGNER		
					VAPEEHPVLLTEAPLNPK		
					DLYANTVLSGGTTMYPGIADR		
					GYSFTTTAER		
					DSYVGDEAQSK		
					DLTDYLMK		
					EITALAPSTMK		
					AGFAGDDAPR		
					AVFPSIVGRPR		
					LCYVALDFEQEMATAASSSSLEK		
					IIAPPER		
					QEYDESGPSIVHR		
					IWHHTFYNELR		
					GILTLK		
					HQGVMVGMGQK		
					VAPEEHPTLLTEAPLNPK		
					LCYVALDFENEMATAASSSSLEK		
					YPIEHGIITNWDDMEK		
					AVFPSMVGRPR		
					VAPDEHPILLTEAPLNPK		
A0A452G9K5	Cytokeratin-1	146	63151.12	8.13	FLEQQNQVLQTK	6.9	Both samples
					TLLEGEESR		
					SLNNQFASFIDK		
					DYQELMNTK		
A0A452G9T9	Alpha-1-B glycoprotein	184	53563.51	5.45	VLSPAGPEAQFELR	39.2	Both samples

					DSAPAELVLSDGTLPAPELSAEPAILSPTPGALVQLR		
					THAAGTPSEPSATVTVEELAPPPAPR		
					ALWTGALTPGR		
					GAEEQLVPR		
					GVTFLLR		
					SELAAWSR		
					CEAQVPDVSFLLLR		
					SADLVLTHVGR		
					ESAQVLRPGSPASLTCVAPLSDVDFQLR		
					AGEEEPLVVAWSTNR		
					SLLSELSDPVELQVAGS		
A0A452GAQ4	Low affinity immunoglobulin gamma Fc region receptor II	75	32952.07	6.44	AVVSIQPAWINVLR	7.4	Colostrum
					ITFYQDGK		
GLCM1	Glycosylation-dependent cell adhesion molecule 1	112	17055.54	5.27	SLFPPASEVVKP	16.9	Both sample
					LMELGHK		
					LPLSILK		
A5JSS8	Alpha-lactalbumin	190	16254.61	5.06	VGINYWLAHK	28.9	Both samples
					FLDDDLTDDIVCAK		
					NICNISCDK		
					LDQWLCEK		

A0A452F7K4	Albumin	381	69173.26	5.80	SLHTLFGDELCK	59.8	Both samples
					MPCTEDYLSLILNR		
					HPYFYAPELLYYANK		
					HGEYGFQNALIVR		
					HLVDEPQNLIK		
					TVMENFVAFVDK		
					HPEYAVSVLLR		
					DVFLGSFLYEYSR		
					YICDHQDTLSSK		
					CCTESLVNR		
					YNGVFQECCQAEDK		
					LKPEPDTLCAEFK		
					APQVSTPTLVEISR		
					ADFTDVTK		
					EYEATLEDCCAK		
					ECCHGDLLECADDR		
					LVASTQAALA		
					ETYGDMADCCEK		
					QTALVELLK		
					YLYEVAR		
					GACLLPK		
					IVTDLTK		
					EDPHACYATVFDK		
					LCVLHEK		
					DDSPDLPK		
					ELTEFAK		

					NCELFEK		
					ECCDKPVLEK		
					NECFLK		
					SHCIAEIDK		
					AWSVAR		
					TCVADESHAGCDK		
					EGCFLLEGPK		
					DAVPENLPPLTADFAEDK		
A0A452E2F6	Alpha-1-acid glycoprotein	115.04	23131.18	5.20	WFYIGSAFR	17.8	Colostrum
					EFLDVIK		
					AIQAAFFYFEPR		
					EYQTIADK		
E9NRZ3	Beta-1 4-galactosyltransferase I	179	44758.33	9.20	GMSVSRPNAVIGK	26.4	Both samples
					FGFSLPYVQYFGGVSALSK		
					VAIIIPFR		
					TTLQGSSHGAAAIGQPSGELR		
					LLNVGFK		
					YSPTDCISPHK		
					YPLYTK		
					YWLYYLHPILQR		
					ITVDIGTPS		
A0A452EH90	Lactadherin	227	47866.36	7.04	INLFDSPLETQYVR	37.7	Both samples
					VTGIITQGAR		

					NIFEVPFQAR		
					IQPVAWHNR		
					VAYSNDGHQFQFIQAAGQLGEK		
					LVPIICR		
					GDVFTHYICK		
					DFGHIQYVAAYR		
					FELLGCELDGCTEPLGLK		
					VELLGC		
					WAPELAR		
					LYQTGIVNAWTSSNYDK		
					IFVGNR		
					VAYSDDGVTWTEYK		
CASB	Beta-casein	305	24865.09	5.26	YPVEPFTESQSLTLTDVEK	72.1	Both samples
					DMPIQAFLLYQEPVLGPVR		
					FQSEEQQQTEDELQDK		
					LHLPLPLVQSWMHQPPQPLSPTVMFPPQSVLSLSQPK		
					IHPFAQAQSLVYPFTGPIPNSLPQNILPLTQTPVVVPPFLQPEIMGVPK		
					GPFPILV		
					EMPFPK		
					VLPVPQK		
CTHL2	Cathelicidin-2	158	19845.83	9.37	LLELDPAPNDEVDPGTR	22.2	
					KPVSFTVK		
					SSEANLYR		
					FRPPIR		

A0A452E537	MAP28 protein	123.5	17745.53	8.85	INEQSSEANLYR	29.1	Colostrum
					MNITCEELQSVGR		
					QCVGTVTLDAVK		
					LLELDPPPK		
A0A452DYF3	BPI1 domain-containing protein	116	17611.48	7.81	TLISSSNENLVQNLK	16	Both samples
					ETLHTAQETVN		
A0A452DYS8	Peptidase S1 domain-containing protein	95	27954.79	10.88	ARPQELPFLASIQNQGR	10.8	Colostrum
					LNDVLLLQLDR		
A0A452DYV8	Ig-like domain-containing protein	161	33821.83	5.56	ESPSVSVTGHAAPGHK	33.4	Both samples
					AYLEEECPGMLQR		
					SIGLHWTR		
					SLAQPLTVPWDPR		
					EGSHTFQGAFGCELR		
					EIPAWVPLDPAAQNTK		
					YLPYSR		
					SSGAFWGYAYDGR		
A0A452E053	Ig-like domain-containing protein	116	11662.05	6.75	DVVLTQTPLSLSVIPGGTASISCK	30.2	Colostrum

					LIYLVSNR		
A0A452E0Z2	Glyceraldehyde-3-phosphate dehydrogenase	241	41963.34	8.13	VPTPDVSVVDLTCR	58.4	Both samples
					SVGSPFVVEATGVYLSLEETK		
					FGILEGLMTTVHSYTATQK		
					AGIALNDNFVK		
					IVSNASCTTNCLAPLAK		
					LISWYDNEYGYSNR		
					GAHQNIIPASTGAAK		
					ELIVGINGFGR		
					LAQPTPYSAIK		
					VVICAPSPDAPMFVMGVNEK		
					VPTPNVSVVDLTCR		
					GAAQNIIPASTGAAK		
					LISWYDNEFGYSNR		
					AITIFQER		
					VIISAPSADAPMFVMGVNHEK		
A0A452E2R6	15 kDa protein B-like	79.64	19564.64	9.87	YDILSNILR	12.8	Colostrum
					GLSYEEIVTQALK		
A0A452E4U5	Antithrombin-III	156	52411.24	6.38	TSDQIHFFFAK	17.4	Colostrum
					NDLYVSDAFHK		
					VTFQANRPFLVLIR		
					LQPLDFK		
					SSELVSANR		

					ANSLFATAFYQHLADSK		
					SPVEDICTAKPR		
A0A452E555	CAP18_C domain-containing protein	144.03	18770.44	8.67	LLELDPPPEQDAEDQGAR	33.9	Colostrum
					SAEANLYR		
					GDFDITCNDIQSVGLFGR		
					TSQQPVEQCDFR		
A0A452E573	ATP synthase subunit beta	227	56183.37	5.14	FLSQPFQVAEVFTGHLGK	39.6	Colostrum
					LVLEVAQHLGESTVR		
					FTQAGSEVSALLGR		
					AIAELGIYPAVDPLDSTSR		
					AHGGYSVFAGVGER		
					IMDPNIVGSEHYDVAR		
					IGLFGGAGVGK		
					VALVYGQMNEPPGAR		
					TVLIMELINNVAK		
					VALTGLTVAEYFR		
					TIAMDGTEGLVR		
					VVDLLAPYAK		
					EGNDLYHEMIESGVINLK		
					IPSAVGYQPTLATDMGTMQER		
A0A452E876	Acrosin-binding protein	179	58736.78	4.99	VASWLQTEFLSFQDGDFPTR	14.7	Colostrum
					FYGLDLYGGLR		

					ICDTEYVQYPNYCAFK		
					FFALLTPTWK		
					LEQCHSEANLQR		
					MDFWCAR		
A0A452E8D3	Ig-like domain-containing protein	151	14523.15	8.93	VSITCSGSSSNIGR	26.9	Both samples
					GYGSWYQQVPGSAPK		
					LLIYGATSR		
A0A452E9L8	Ig-like domain-containing protein	148	14352.77	6.32	VSITCSGSNIGSSGVGWYQQLPGSGLK	31.2	Colostrum
					TVIYYNSNRPSGVPDR		
A0A452ED43	Apolipoprotein A4	158	42858.49	5.30	SLAPYAQDVQGK	38.2	Colostrum
					LGPLAGDVEDHLSFLEK		
					AMVQQLDALR		
					LGPYAEELR		
					LVPFATELHER		
					TVGPYGETFNK		
					GHLTPYADQLQTK		
					LLPHATEVSQK		
					LGEVSTYTDDLQK		
					SELTQQLNTLFQDK		
					TQVDTQAQQLR		
					LNHQLEGLAFQMK		

A0A452EDG7	Polymeric immunoglobulin receptor	260	82601.96	5.80	DNGVFSVHIASLR	32.2	Both samples
					SNVYLQVLEPEPELVYR		
					TVTINCPFTSANSQMR		
					SVSITCYYPATSVNR		
					SSVTFDCSLGPEVADTAK		
					NGEACNVVINTLGK		
					GGSVTVSCPYNPK		
					VVEGEPSLK		
					SPIFGPEEVTSVEGR		
					CGLGISSR		
					DAGMYVCQAGDDAK		
					QVNAAPAGGAIESR		
					TGQGCFLIIDSTGYK		
					FYSFEK		
					CTTLISSEGYVSDDYVGR		
					LSCHFPCK		
					ILFLPK		
					EDEGWYWCGVK		
					YGETAAVYVAVESR		
A0A452EE69	Ig-like domain-containing protein	100	14513.70	5.55	VDAEVWK	31.3	Both samples
					WLQGNQELPR		
					YLTWGPLPEPGQSVATFAVTSVLR		

A0A452EEM4	Peptidoglycan-recognition protein	205	21050.07	9.37	YVVVSHTAGSVCDTPASCLR	34.2	Both samples
					AAQNLLACGAAR		
					DVQQTLSPGDQLYDIIQQWPHYR		
					GYLTPNYEVK		
A0A452EH63	Fructose-bisphosphate aldolase	178	39436.12	8.45	IGEHTPSSLAIMENANVLAR	30.2	Both samples
					YSHEEIAMATVTALR		
					GILAADESTGSIAK		
					GVVPLAGTNGETTTQGLDGLSER		
					CPLLKPWALTFSYGR		
					ADDGRPFPQVIK		
					ALANSLACQGK		
A0A452EHJ9	Inter-alpha-trypsin inhibitor heavy chain 4	90	99397.51	5.75	GESAGLVTATGR	2.5	Colostrum
					FLAPPPPFYR		
A0A452EHZ1	40S ribosomal protein S27a	178	17964.92	9.68	TITLEVEPSDTIENVK	21.8	Colostrum
					TLSDYNIQK		
					ESTLHLVLR		
A0A452EIH8	Nucleobindin-1	199	54653.15	5.14	LVTLEEFLASTQR	28.2	Both samples
					DLELLIQTATR		
					LQAANAEDIK		
					VNVPGSQAQLK		

					LSQETEALGR		
					ELQQAVLQMEQR		
					YLESLGEEQR		
					YLQEVINVLETDGHFR		
					DLAQYDAAHHEEFK		
					ELDFVSHHVR		
					MPPSGPRA		
					FEEELAAR		
A0A452EKB3	Immunoglobulin gamma 2 heavy chain	196	35578.10	6.20	TFPAVLQSSGLYSLSSVVTVPASTSGAQTFICNVAHPASSTK	37.4	Both samples
					VHNEGLPAPIIR		
					VVSALPIQHK		
					SSWQEGDTYTCAVMHEALR		
					EPQVYVLAPPR		
					DWLQGK		
					VDKPVSQVQNPHR		
					EEQFNSTFR		
A0A452EN73	Fibrinogen gamma chain	182	50405.32	5.78	IHLISTQSTIPYVLR	31.2	Both samples
					ISYNPDEPSKPSNIESATK		
					TSEATELVK		
					ESGLYFIRPLK		
					TSTADYASFK		
					FLQEIYNSNNQK		
					LAIDGQQHHLGGAK		

					IIPFNR		
					EGFGHLSPTGNTEFWLGNEK		
					YETLISNHESTIR		
					IQLEDWNGR		
A0A452EN82	ATP synthase subunit alpha	235	60792.01	9.20	ILGADTSVDLEETGR	31.3	Colostrum
					EVAAFAQFGSDLDAATQQLLSR		
					TGAIVDVPVGEELLGR		
					GIRPAINVGLSVSR		
					TGTAEVSSILEER		
					HALIIYDDLSK		
					EAYPGDVFYLHSR		
					VLSIGDGIAR		
					GMSLNLEPDNVGVVVFGNDK		
					AVDSLVPIGR		
					TSIAIDTIINQK		
					VVDALGNAIDGK		
					ELIIGDR		
A0A452ENB6	Complement component C9	214	61975.42	5.64	TENHEEEIQVLR	17.2	Both samples
					LAPIYDLIPVK		
					VVEESELAR		
					LAAEATFR		
					AIEDYINEFSSR		
					KPWNVASLSYDTK		
					YTPVEAIEK		
					LLSSYSAK		

					AGVEVTGNVDSK		
A0A452EPC6	Immunoglobulin mu heavy chain	288	52990.67	6.50	IVSDISEGQVETVQSSPITYR	24.8	Both samples
					VFPLVSCVSSPSDENTVALGCLAR		
					ALETHTYFER		
					SSLICQATDFSPK		
					GGKPVGTVMVVAPK		
					DFVPNSVSFSWK		
					FWTFPEVLR		
					AYSMLTITER		
					QISLSWFR		
					EWLSQSAYTCQVEHNK		
A0A452EPW9	CD5 antigen-like	110	39360.41	5.98	GTPSGNLYKPLADEK	6.7	Colostrum
					AGWNLSAAK		
A0A452EQL8	Pentaxin	121	25046.61	8.32	GEVFVKPQLW	25.3	Colostrum
					ETENSYVSLK		
					RSYSIFSYATR		
					AFVFPK		
					EASIILGQEQDAFAGGFDR		
A0A452EU19	Alpha-2-macroglobulin	128.25	164249.41	6.02	ASFSVLGDILGSAMR	7582.9	Colostrum
					SNSFVYLEPLPR		
					ICPQPEQYR		
					DTGLGLSPTASLR		
					ALLAYAFALAGNQER		

					GIPFTGQVLLVDGK		
					SLFTDVVAEK		
					IEVEAK		
					LLIYAILPDGEVVGDSAR		
A0A452EU55	2-phospho-D-glycerate hydro-lyase	193	47312.06	6.17	AAVPSGASTGIYEALELR	34.8	Both samples
					GNPTVEVDLFTAK		
					DYPVVSIEDPFDQDDWEAWQK		
					IGAEVYHNLK		
					LAMQEFMILPVGAENFR		
					YNQILR		
					YITPDELADLYK		
					TIAPALVSK		
					VNQIGSVTESLQACK		
					IEEELGSK		
					SGETEDTFIADLVVGLCTGQIK		
					VVIGMDVAASEFYR		
A0A452EVZ5	Ig-like domain-containing protein	141	16603.78	6.05	HKPGQSPEGLIYQVSNR	25.8	Colostrum
					LTGSGSGTDFTFTISR		
					TYLNWIK		
A0A452EZ30	Leucine rich alpha-2-glycoprotein 1	195	39058.05	6.76	FLEASWLHGLK	24.1	Both samples
					FLLPVPQLK		

					LQVLGEGLLAPQPK		
					GPLNLER		
					ISAALGTLVLK		
					LASVAAGAFR		
					YLFLNDNR		
					LPPGLFR		
					LLAVAESH		
A0A452EZW6	Xanthine dehydrogenase/oxidase	196	146646.25	8.38	IPAFGSIPTEFR	10594	Both samples
					VLFQPGSTQVK		
					VTYEDLPAIITIEDAIK		
					VSLSATGFYR		
					GEAGEMELFASTQNPMK		
					TNLPSNTAFR		
					STLVTVAVALAAYK		
					LEGFSVPR		
					LDSPATPEK		
					YESELFLR		
					VTWIQSSTLK		
					GVLEQLR		
					GLCIIPTK		
A0A452F1G2	Thrombospondin 1	153.63	134213.49	4.78	FVFGTTPEDILR	8374.4	Colostrum
					FTGSQPFGR		
					FQDLVDAVR		
					AQGYSGLSVK		

					NALWHTGNTPGQVR		
					GGVNDNFQGVLQNVR		
					TIVTTLQDSIR		
					QVTQSYWDTNPTR		
					GFLLLASLR		
A0A452F257	Matrix Gla protein	86	11977.83	9.71	YAMVYGYNAAYDR	23.8	Colostrum
					NANTFISPQQR		
A0A452F4L3	Fibrinogen alpha chain	194	66999.17	6.52	ANNNDNTFNQVSEDLR	18.9	Both samples
					GLIDEVNQDFTSR		
					MSAVTGPVPR		
					TGLAPESPR		
					QLEQVIAINLLPSR		
					DTQYLSLIK		
					NIVEILR		
					VIEQAQHINLLQK		
					GNLEDFFHR		
					LESDVPDLR		
					ELLIGNEK		
A0A452F548	SERPIN domain-containing protein	167	43674.22	5.98	VLELPYEGK	34.6	Both samples
					IPELLASGMVDSLTK		
					SYDFLPEFLASTQK		
					FAVDLFR		
					IEQQLTLEK		

					FMVEATK		
					ADLSGMSGASDLFISK		
					LVLVNAIYFK		
					MYNAELASVDFLR		
					VLHFEGVEIHSGFQSLNADINK		
					LEESYDLSEPLAR		
A0A452F6Q6	Ig-like domain-containing protein	85	13819.61	7.88	LHTDVPSR	12.7	Colostrum
					LLIYYATR		
A0A452DZD6	Ig-like domain-containing protein	221	11298.58	8.80	SSYSCEVTHEGSTVK	75.5	Both samples
					SAPSVTLFPPSTEELNANK		
					YAASSYLTLTGSEWK		
					ADGSTINQNVK		
					ATVVCLISDFYPGSVTVVWK		
A0A452E2C0	Transthyretin	171	13633.36	5.58	HYTIAALLSPYSYSTTALVSSPK	59.1	Both samples
					AADETWEPFASGK		
					GSPAANVGVK		
					VLDAVR		
					SLGISPFHEYAEVVFTANDSGLR		
A0A452E2P1	Immunoglobulin alpha heavy chain	166	34884.60	8.38	SAEGASFTWSPTGGK	18.3	Both samples

					TVDVPCK		
					FVTCQVQHLSK		
					NFPAVLDGNQYTMSSQLTLPASLCPENK		
A0A452E2Y0	Clusterin	223	50892.66	5.77	RPQDTQYYSPFSSFPR	34.6	Both samples
					LYDQLLQSYQQK		
					LFNSLPITVTVPQEVSNPNFMENVAEK		
					ASSIMDELFQDR		
					TQIEQTNEER		
					GSLFFNPK		
					QAQQAMDAHLQR		
					MLDTSALLK		
					LLLSSLEEAK		
					TPYHFPVTEFTENNDR		
					CQEILEVDCSASSPTQTLLR		
A0A097C2Z1	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide (Fragment)	133	27745.10	4.73	SVTEQGAELSNEER	27.3	Colostrum
					FLIPNASQAESK		
					DICNDVLSLLEK		
					DSTLIMQLLR		
					YLAEVAAGDDK		

					NLLSVAYK		
A0A452DPJ3	Ig-like domain-containing protein	109.17	17025.58	6.70	SGNTATLTISGLQAEDEADYYCSSYK	20.4	Colostrum
					TLIYNLNK		
A0A452DWD6	Zona pellucida binding protein	151	39559.78	9.08	FFNQQVEVLGR	18.3	Colostrum
					SPHVLCVTQR		
					YHAAPCNSIYNISFEK		
					CPECCVICSPGSYNSR		
					EPHYYYQFTAR		
A0A452DWU1	Histone H4	110	11367.34	11.36	DAVTYTEHAK	38.8	Colostrum
					VFLENVIR		
					DNIQGITKPAIR		
					ISGLIYEETR		
A0A452ECU4	Complement C1s	97	77852.47	4.96	VEDPESTLFGSITR	8.6	Colostrum
					VFIHPGWEVLDSSITR		
					IFGGSIAK		
					IENFPWQVFFSNPR		
					VLGTELPK		
A0A452EDZ4	Protein S100	60.3	10890.42	5.45	LGHYDTLVK	20.7	Colostrum
					ELPNCIQNTK		

A0A452ET55	Triosephosphate isomerase	181	31471.72	5.71	VVLAYEPVWAIGTGK	52.9	Colostrum
					SNVSDAVAQSAR		
					VPADTEVVCAPPTAYIDFAR		
					IIYGGSVTGATCK		
					HVFGESDELIGQK		
					VAHALAEGLGVIACIGEK		
					DLGATWVVLGHSER		
					ELASQPDVDGFLVGGASLKPEFVDIINAK		
					FFVGGNWK		
					NNLGELINTLNAAK		
A0A452ET82	Pyruvate kinase	132	58091.07	7.24	NTGIICTIGPASR	29.6	Colostrum
					IYVDDGLISLLVK		
					GDLGIEIPAEK		
					LDIDSPPITAR		
					GDYPLEAVR		
A0A452EW27	Inhibitor of carbonic anhydrase	217	77608.39	6.37	YLGPEYLQAIANVR	28.7	Both samples
					DLLFTDAATGFLR		
					FFASSCVPCADQSNFPK		
					NLKPEDFQLLCLDGSR		
					SDADLTWNSLR		
					KPVTEAQSCHLAVVPSHAVVSR		
					AVPEAEGPQR		
					LCQLCAGK		

					VPSHAIVAR		
					VLSVDGPHVACVK		
					GDVAFVK		
					DLLFSDDTECLANLQDR		
					SAEFQLFYSPHGK		
					MLFNQQELFGR		
					GYYVVAVVK		
					GSDFQLNQLQGK		
A0A452EYF6	Heat shock protein family A (Hsp70) member 8	135	70508.70	5.32	VEIIANDQGNR	5	Colostrum
					DAGTIAGLNVLR		
					LLQDFFNGK		
A0A452F924	F5/8 type C domain-containing protein	97	130971.76	5.08	TPSQEQLLAAAMAAAR	4909.6	Colostrum
					YLSPDATVSTEVR		
					ILNPGEYR		
					GEDEEEASEAQETPDHAIFR		
A0A452FKE5	C4a anaphylatoxin	112.15	190810.22	8.05	VTASDPLEALGSEGALSPGGLASLLR	4118.3	Colostrum
					FVSSPFSLDLSK		
					DGSYGAWLHR		
					DSSTWLTAFVLK		
					STGLCVATPAR		

A0A452FM11	Tubulin beta chain	147.9	50017.17	4.77	GHYTEGAELVDSVLDVVR	20.9	Colostrum
					YLTVAAVFR		
					MSATFIGNSTAIQELFK		
					LTTPTYGDLNHLVSATMSGVTTCLR		
					ISEQFTAMFR		
					NSSYFVEWIPNNVK		
A0A452FXA4	Melanotransferrin	62.41	80663.93	5.81	TVGWNVPVGYLVDSGR	3.1	Colostrum
					ADVTEWR		
A0A452FXZ3	Apolipoprotein H	58.55	39367.19	8.53	TCPKPDELPFSTVVPLK	7.3	Colostrum
					ATVIYEGER		
A0A452G077	Cellular communication network factor 2	101.88	38053.31	8.41	LPSPDCPFPR	12.3	Colostrum
					TTTLPVEFK		
					VTNDNAFCR		
					ANCLVQTTEWSACSK		
A0A452G5R1	Haptoglobin	143	44657.91	8.10	VTSILDWVR	23.7	Colostrum
					DIGLQLPECEEDVSCPEAPK		
					NVGQQLPECEAVCGKPK		
					NQLVEVEK		
					YVMLPVADQDK		
					TATSPVGVQPILNENTFCVGLSK		
					DITPTLR		

A5YU17	Growth/differentiation factor 8	166	42827.35	7.01	ALDENGHDLAVTFPEPGEEGLNPFLEVK	34.7	Colostrum
					DFGLDCDEHSTESR		
					ELIDQYDVQR		
					TVLQNWLK		
					ANYCSGECEFLFLQK		
					TPTTVFVQILR		
					IPGMVVDR		
					LDMNPGTGIWQSIDVK		
					EQIIYGK		
					YPHTHLVHQANPK		
LACB	Beta-lactoglobulin	319	19975.53	5.50	VAGTWYSLAMAASDISLLDAQSAPLR	73.9	Both samples
					YLLFCMENSAEPEQSLACQCLVR		
					VYVEELKPTPEGNLEILLQK		
					LAFNPTQLEGQCHV		
					IDALNENK		
					VLVLDTDYK		
					IPAVFK		
					ALPMHIR		
					IIAEK		
					WENGECAQK		
					GLDIQK		
A0A452DLU0	Hexokinase	186	105040.75	6.48	LSDETLLDIMNR	16.8	Colostrum
					ATDCVGHDVATLLR		

					TTVGVDGSLYK		
					GAALITAVGVR		
					GAAMVTAVAYR		
					NILIDFAK		
					SANLVAATLGAILSR		
					EGLLFEGR		
					LPVGFTFSFPCR		
					GDFIALDLGGSSFR		
					QIEETLAHFSLTK		
					AILQQLGLNSTCDDSILVK		
					HIDLVEGDEGR		
A0A452DP35	L-lactate dehydrogenase	138	36550.53	7.14	VIGSGCNLDSAR	49.4	Both samples
					SADTLWGIQK		
					LVIITAGAR		
					LLVVSNPVDILTYVAWK		
					QVVDSAYEVIK		
					FIIPNIVK		
					EVVGSAYEIIK		
					LGVHPSSCHGWIIGEHGDSSVPLWSGVNVAGVALK		
					MLIVSNPVDILTYVVWK		
					GETMDLQHGSLFFNTPK		
					FIIPQIVK		
					GLTSVINQK		

A0A452DPJ7	Fumarate hydratase mitochondrial	110	54621.96	8.97	IYELAAGGTAVGTGLNTR	12.2	Colostrum
					THTQDAVPLTLGQEFSGYVQQVK		
					ATAIELGYLTAEQFDEWVKPK		
A0A452DPV3	Phosphoglycerate kinase	170	44700.99	8.61	LGDVYVNDAFGTAHR	22.8	Colostrum
					GCITIIGGGDTATCCAK		
					ALENPERPFLAILGGAK		
					VLNNMEIGASLFDEEGAK		
					VLPGVDALSSV		
					ALESPERPFLAILGGAK		
A0A452DQM6	60 kDa chaperonin	118	60963.52	5.71	VGLQVVAVK	11	Colostrum
					LVQDVANNTNEEAGDGTTTATVLAR		
					TVIIEQSWGSPK		
					AAVEEGIVLGGGCALLR		
A0A452DQX3	Protein piccolo-like	82	129175.69	6.41	LGVQPEELAVLVADSPISPR	4152.8	Colostrum
					TTGLVLGSGHGTSYVAPIVTGDLAPLDTYR		
A0A452DTD2	Transket_pyr domain-containing protein	65	117667.32	6.38	FGLEGCEVLIPALK	4335.2	Colostrum
					GHHVAQLDPLGILDADLDSSVPADIISSTDK		
A0A452DTF9	C-type lectin domain-containing protein	109	22070.29	5.47	TFHEASEDCISR	28.7	Colostrum

					TQLDSLAQEVALLK		
					EQQALQTVCLK		
					GGTLGTPQTGSENDALYEYLR		
A0A452DVP7	Lymphocyte cytosolic protein 1	102	70179.19	5.17	VYALPEDLVEVNPK	9.1	Colostrum
					LNLAFIANLFNK		
					FSLVGIGGQDLNEGNR		
					VNDDTIVNWVNETLK		
A0A452DVV1	Cysteinylglycine-S-conjugate dipeptidase	135	56196.27	6.07	LILADALCYAHTFNPK	12.9	Both samples
					FAEIVEENLK		
					GVLFASGQNLAR		
					TIQVDNTDAEGR		
					GLVLGIYSK		
					TDVFIRPK		
A0A452DW73	Aconitate hydratase mitochondrial	148	85401.40	7.87	IVYGHLDDPANQEIER	11.8	Colostrum
					NDANPETHAFVTSPEIVTALAIAGTLK		
					DVGGIVLANACGPCIGQWDR		
					SQFTITPGSEQIR		
					DINQEVYNFLATAGAK		
A0A452DYD2	ATP synthase subunit	63	11416.47	9.60	APALVNAAVTYSKPR	23.3	Colostrum
					LATFWYYAK		

A0A452DZE4	ACB domain-containing protein	58	9788.36	7.64	QATQGDCNIPAPPATDLK	29.9	Colostrum
					LLVYSYYK		
A0A452E0A1	Glutathione S-transferase	176	27491.97	8.70	MQLVQLCYNPDHEK	42.2	Colostrum
					SMVLGYWDIR		
					LTFVDFLTYDVLDQNR		
					YLEQLPGQLK		
					MLLEFTDTSYEEK		
					LTQSNAILR		
					QFSLFLGK		
					CLDEFPNLK		
					IATYMQSDR		
A0A452E0N8	Chromosome 15 open reading frame 48	61	9633.45	9.89	LVTINQEWKPIEELQK	37.3	Colostrum
					NPEPWETVDPTVPTK		
A0A452E1T9	BTB domain-containing protein	182	66861.85	8.51	VVISEQNVEELLR	23.3	Colostrum
					TLAPPPEALDCPACCLAK		
					FFNYINLNAVSNK		
					NYNSFVLQNLNK		
					AAALSATSAGR		
					GALLDSVVILGGQK		
					DYTINPNAYLLDQK		

					GAQYFNTPR		
					YIYISGGTTEQISGLK		
					NLYIVTGR		
					QSVEINLQK		
A0A452E294	Superoxide dismutase	100	24579.96	8.89	AIWNVINWENVTAR	13.1	Colostrum
					HHAAYVNNLNVAEEK		
A0A452E2H1	ATP synthase peripheral stalk subunit OSCP	86	21456.29	9.71	YATALYSAASK	14.3	Colostrum
					FSPLTSNLINLLAENGR		
A0A452E495	Heat shock-related 70 kDa protein 2	133	69824.81	5.50	VEIIANDQGNR	10.4	Colostrum
					IINEPTAAAIAYGLDK		
					VQSAVITVPAYFNDSQR		
					DAGTITGLNVLR		
					FEELNADLFR		
A0A452E4F5	RNAse_Pc domain-containing protein	66	18554.48	9.41	FNTFIHEDLWNIR	14.7	Colostrum
					SICSTTNIQCK		
A0A452E4Y9	Glycerol kinase	141	60834.89	5.63	GIICGLTQFTNK	8.8	Colostrum
					TGLPLSTYFSAVK		
					LQELNIDISNIK		
					QILQSVNECIEK		

A0A452E570	TIP120 domain-containing protein	68	136403.78	5.52	SVILEAFSSPSEEVK	2195.1	Colostrum
					LGTLSALDILIK		
A0A452E5B4	Peroxiredoxin-5	90	22457.18	8.70	FSMVIEDGIVK	12.7	Colostrum
					GVLFGLPGAFTPGCSK		
A0A452E712	Adenylate kinase isoenzyme 1	107	22457.18	8.70	YGYTHLSTGDLLR	12.9	Colostrum
					IIFVVGGPGSGK		
A0A452E7A0	Plasminogen	93	91190.87	7.51	IFTPETNPR	2.8	Colostrum
					EASVQEIPVSR		
					IGNGK		
					LPVIENK		
A0A452E7L5	Sperm acrosome associated 9	89	20397.33	7.21	IFQQQQFTFIGALEHCR	21.3	Colostrum
					LCQHFEALHAGTPVTNNLLEK		
A0A452E968	T-complex protein 1 subunit gamma	106	60585.89	6.38	TAVETAVLLLR	6.2	Colostrum
					IVLLDSSLEYK		
					TLIQNCGASTIR		
PERL	Lactoperoxidase	277	80365.85	8.71	VGPLLACLLGR	25.6	Both samples

					IHGFDLAAINLQR		
					DQINAVTSFLDASLVYGSEPSLASR		
					AGFVCPTPPYQSLAR		
					FGHMEVPSTVSR		
					ASEQILLATVHTLLLR		
					LDLSPWASR		
					ISNVFTFAFR		
					GFCGLSQPK		
					DGGIDPLVR		
					LICDNTHVTK		
					IVGYLDEEGVLDQNR		
					GLQAVLK		
					FWWENPGVFTEK		
					LLDLYK		
A0A452EAI1	IZUMO family member 4	113	24079.51	6.03	ELHLAIPAEITR	17.5	Colostrum
					EQVHLIQNAIIESR		
					LNQVANAVYQR		
A0A452EAT2	Angiotensin-converting enzyme	161	150584.13	6.08	ENYNQEWWSLR	8149.3	Colostrum
					GPQFGSEVELR		
					NYQDLAWAWK		
					FHIPASVPYVR		
					VFDGSVTR		
					YQGVCPPLAR		

					LNGYQDGGDSWR		
					EGANPGFHEAIGDVLALSVSTPTHLHK		
					DLPVTFR		
A0A452EB34	ADP/ATP translocase	112	32858.33	9.78	DFLAGGIAAAISK	46.6	Colostrum
					YFPTQALNFAFK		
					EQGVLSFWR		
					LLLQVQHASK		
					YFAGNLASGGAAGATSLCFVYPLDFAR		
					IYQQEGIGAFFR		
					GTGGALVLVLYDK		
					EQGFLSYWR		
					DLLAGGVAAAVSK		
					LLLQVQASSK		
					ASYFGAYDTVK		
A0A452EC76	Zonadhesin	88	272753.34	6.85	FVQLQTAFGLR	1644.6	Colostrum
					VTLPAIPSGGVFLTPSGR		
					VTAPYEPVDQLR		
A0A452ECF9	Radial spoke head 6 homolog A	100	80335.60	4.33	ILNQRPEDPLFLLESLNR	4.4	Colostrum
					LPHVTPIQIVQAR		
A0A452EDB8	Mitochondrial carrier 2	98	32161.65	8.70	VLIQVGYEPLAPTVGR	10.9	Colostrum
					SAATLITHPFHVITLR		
A0A452EFQ1	Tektin	128	56659.97	6.53	LVNEVYEVDDTIQTLQQR	14.5	Colostrum

					SNLTNFQESNTSR		
					VDATVSVPESWAK		
					ATLEHDLAVK		
					YTPDDWYR		
					AIAQLAANR		
A0A452EGC2	Voltage-dependent anion-selective channel protein 2	148	30971.85	6.37	YQLDPTASISAK	28.1	Colostrum
					LTLSALVDGK		
					VNNSSLIGVGYTQTLRPGVK		
					GFGFGLVK		
					NNFAVGYR		
					TGDFQLHTNVNDGTEFGGSIYQK		
A0A452EHI7	Uncharacterized protein	78	21844.20	5.57	LVIVGDGACGK	10.4	Colostrum
					TCLLIVFSK		
A0A452EJS2	Platelet-activating factor acetylhydrolase	89	50004.03	5.90	IPQPLFFINSER	5.9	Colostrum
					IQALMAAANIGQSK		
A0A452EJT8	Amino_oxidase domain-containing protein	160	57938.19	6.38	DALSDLALHFLNK	19.2	Colostrum

					IGLIQFCLSAPK		
					SIHDALCVIR		
					VIDPATATSVDLR		
					TLSGMESYCIR		
					GIHPTIISESFQK		
					TVTIVVR		
					TGPPAGAVGAAGAAGGR		
					AYILNLVK		
A0A452EK10	Amino_oxidase domain-containing protein	165	62235.67	9.68	LALDDVAALHGPIAYR	18.3	Colostrum
					HTLLEYLLGEGNLSQPAVR		
					IYFAGEHTAYPHGWVETAVK		
					ALLSSLSGPVLLHAPVVAIK		
					IVGGWDLLPR		
					TGWIGELGAMR		
					VFLSFR		
A0A452EN63	Fibrinogen beta chain OS=Capra hircus OX=9925 GN=FGB PE=4 SV=1	137	54468.40	7.11	DNENVVNEYSSHLEK	20.8	Colostrum
					GGWTVIQNR		
					SILEDLR		
					TPCTVTCNIPVVSGK		

					KPPDADGCLHADPDLGVLCPTGCQLQDTLVK		
					IRPYFPEQ		
					YQLSVSK		
					ECEEIIR		
A0A452ENI4	Rhophilin associated tail protein 1	184	23933.62	5.29	VLNLPTDLFNSVMNVGR	48.1	Colostrum
					MLNYIEQEVIGPDGLIK		
					IVCEVLSSDHDSGPPR		
					VALSNWAELTPELLK		
					LIVHADELAQMWK		
					VNDFTQNPR		
					FLALACSSLGVTIAK		
A0A452EQ11	Cytochrome b-c1 complex subunit 1. mitochondrial	127	52867.72	5.90	MVLAAAGGLEHR	17.3	Colostrum
					YFYDQCPAVAGFGPIEQLPDYNR		
					NALVSHLDGTTPVCEDIGR		
					DVVFNYLHATAFQGTPLAQSVEGPSENVR		
A0A452ESF6	Actinin alpha 1	94	102733.49	5.36	VGWEQLLTTIAR	2.5	Colostrum
					LASDLLEWIR		
A0A452EUU0	Glucose-6-phosphate isomerase	108	102733.49	5.36	HFVALSTNTAK	7.7	Colostrum
					TLATLNPESSLFIIASK		
					ILLANFLAQTEALMR		

A0A452EVX4	CCT-alpha	77	60294.53	5.70	ICDDELILIK	5	Colostrum
					EQLAIAEFAR		
					TSASVILR		
A0A452EX74	A-kinase anchoring protein 4	225	92889.21	6.24	EIVSDLIDSCMK	26.8	Colostrum
					TFLYSELSNK		
					YALGFQHALSPSASSCK		
					VHSSGKPIPACVVLK		
					GYSVGDLLQEVMK		
					LDMSNMVLSLIQK		
					NLHNITGVLMTDSDFVSAVK		
					LSSLVIQMAR		
					LVSALLGEK		
					TEGSVCLFK		
					LCLIMAK		
					LLNESPFNCEDLCEGENK		
					QFIDQLVESVMK		
					IASEMAHDAVEVTSAEMR		
					ESLTMWNQK		
					QLDEAVGNMAR		
					ISPSTDSLAK		
					QNAADIMEAMLK		
A0A452EY47	Lactotransferrin	364	82835.69	8.48	LRPVAAEIYGTEK	54	Both samples
					GEADALSLDGGYIYTAGK		

AAHVEQVLLHQQALFGK
LLCLDGTTKPVTEAQSCYLAVAPNHAVVSR
SPQTHYYAVAVVK
YSSLDCVLRPTEGYLAVAVVK
SSLCALCAGDDQGLDK
VDSALYLGSR
NLLFNDNTECLAK
ECHLAQVPSHAVVAR
FFSASCVPCVDGK
VVWCAVGPEEQSK
YYGYTGAFR
LGAPSITCVR
CSTSPLLEACAFLTR
FQLFGSPEGR
APVDAFK
ANEGLTWNSLK
TSALECIR
YLGTEYVTAIANLK
AYPNLCQLCK
ETTVFENLPEK
ENLIWELLR
CACSSQEPYFGYSGAFK
DSALGFVR
CLQDGAGDVAFVK
WCAISLPEWSK
GSNFQLDQLQGQK

					NCPDQFCLFK		
					YLTALK		
					NADAVTLDSGMVFEAGLDPYK		
A0A452EYS8	Prohibitin	140	32343.30	9.80	IGGVQQDTILAEGLHFR	13.8	Colostrum
					LLLGAGAVAYGIR		
					FNASQLITQR		
A0A452EZP1	Alpha-mannosidase	187	115633.93	5.97	ILHDVELLSSLALAR	15192	Colostrum
					LFGDSCPVAELSSFLTPER		
					KPVLGQAGTLAVGTEGGVR		
					GNVLSLSLLR		
					VELTIPEAWVGQEVHLR		
					LTSLVLVASGR		
					FVSPLYFTDCNLR		
					LSQEVVLDVGCPYVR		
					FEVWAHR		
					AELAVFHR		
					TNHSAFLFGFGDGGGGPTQTMVDR		
A0A452F014	SERPIN domain-containing protein	90	45366.65	9.01	STHEQDFYVTPETVVR	40.4	Colostrum
					TLYLADTFPTDFENPEGAK		
					INDYVEK		
A0A452F044	Myeloperoxidase	96	82485.46	9.67	VGPLLACLIGTQFR	4.9	Colostrum
					VVLEGGIDPILR		

					IANVFTNAFR		
A0A452F093	Fructose-bisphosphatase	78	38210.08	6.16	DFDPALTEYVQR	8.3	Colostrum
					GTGEMTQLLNSLCTAVK		
A0A452F0F0	Cytochrome c domain-containing protein	127	35310.77	9.14	HGGEDYVFSLLTGYCEPPTGVSLR	20	Colostrum
					LSDYFPKPYPNPEAAR		
					GLLSSLDHTSIR		
					HLVGVCYTEDEAK		
A0A452F194	RAB2B. member RAS oncogene family	169	24167.38	6.59	EHGLIFMETSAK	83.3	Colostrum
					YIIIGDTGVGK		
					LQIWDTAGQESFR		
					GAAGALLVYDITR		
					ETFNHLTSWLEDAR		
					FQPVHDLTIGVEFGAR		
					IGPQQCISTPVGPSASQR		
					SCLLLQFTDK		
					TACNVEEAFINTAK		
					DTFNHLTTWLEDAR		
					TASNVEEAFINTAK		
					IQEGVFDINNEANGIK		
					QHSNSNMVIMLIGNK		

A0A452F2G7	Succinate dehydrogenase [ubiquinone] flavoprotein subunit mitochondrial	117	72974.67	6.89	LGANSLLDLVVFGR	8.4	Colostrum
					AAFGLSEAGFNTACVTK		
					NTVIATGGYGR		
					GVIALCIEDGSIHR		
A0A452F509	ATP synthase subunit gamma	122	33086.03	9.34	VYGVGSLALYEK	15.1	Colostrum
					HLIIGVSSDR		
					GLCGAIHSSVAK		
					THSDQFLVTFK		
A0A452F873	Cytochrome c oxidase subunit	84	10519.12	9.54	VYHSLCPISWVQR	27.3	Colostrum
					NCYQNFLDYHR		
A0A452FAP5	Succinate--CoA ligase [GDP-forming] subunit beta mitochondrial	84	46663.97	7.51	LEGTNVHEAQNILSNSGLPITSAVDLEDAAK	9.5	Colostrum
					INFDDNAEFR		
A0A452FBM2	75 kDa glucose-regulated protein	87	73707.57	5.97	AQFEGIVTDLIR	5.6	Colostrum

					DAGQISGLNVLR		
					TTPSVVAFTADGER		
A0A452FFW1	Voltage-dependent anion-selective channel protein 3	151	30738.74	8.95	VNNASLIGLGYTQTLRPGVK	26.1	Colostrum
					LTLSALIDGK		
					VGLGFELEA		
					LTLDTIFVPNTGK		
					LSQNNFALGYK		
					ICNYGLTFTQK		
A0A452FIF4	Tektin	127	56487.87	8.12	LVNEVFTIDDTLQTLK	12.7	Colostrum
					LQLCGAEASR		
					IGIDLVHDNVEK		
					DTLQLLVMTK		
					NTSDCISFFHGVEK		
A0A452FL64	Resistin	72	11534.50	8.29	NIGLDCQSVTSR	23.9	Colostrum
					IQDVTTSLVPEAVR		
A0A452FLG6	Deoxyribonuclease	127	35147.38	9.30	DFVIVPLHTTPETSVR	16.4	Colostrum
					EIDELADVYTDVK		
					ALDVSDHFPVEFK		
					EQYAFLYK		

A0A452FMZ7	HATPase_c domain-containing protein	166	84730.75	4.92	NPDDITNEEYGEFYK	16.2	Both samples
					HIYYITGETK		

GVVDSEDLPLNISR

					SLTNDWEDHLAVK		
					HLEINPDHSIIETLR		
					DQVANSAFVER		
					ALLFVPR		
					TLTIVDTGIGMTK		
					ADLINNLGTIAK		
					YIDQEELNK		
A0A452FNC0	Saccharopine dehydrogenase (putative)	107	47304.65	9.15	GVYIIGSSGFDSIPADLGVIYTR	8.4	Colostrum
					QATVVLNCVGPYR		
A0A452FPW8	Enoyl-CoA hydratase	114	83294.65	9.20	TVLGSPEVLLGILPGAGATQR	7.1	Colostrum
					DTTASAVDVGLR		
					GFYIYQEGVK		
					MQLLEIITTEK		
A0A452FQM6	NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial	73	50619.81	8.51	HAGGVTGGWDNLLAVIPGGSSTPLIPK	9.3	Colostrum
					GDARPAEIDSLWEISK		
A0A452FRY4	Acrosin	124	45704.46	9.58	RPGVYTSTWSYLNWIASK	9.4	Colostrum
					STNVCAGYPEGK		
					LQQLIEVLK		
A0A452FSH7	Protein kinase cAMP-dependent	99	45144.98	4.80	GQYFGELALVTNKPR	13	Colostrum

	type II regulatory subunit alpha				VDEHVIDQGDDGDNFYVIER		
					AATIVATSEGSLWGLDR		
A0A452FTD4	Tektin	104	50606.81	6.10	NTGAHTSSGLATSGFR	6.6	Colostrum
					YLLDEWFQNCYAR		
A0A452FXX0	Malate dehydrogenase	192	35577.43	8.82	SQETDCPYFSTPLLLGK	38.5	Colostrum
					LTLYDIAHTPGVAADLSHIETR		
					IFGVTTLDIVR		
					TIIPLISQCTPK		
					GCDVVVIPAGVPR		
					EGVVECSFVK		
					IQEAGTEVVK		
					MIAEAIPELK		
					AGAGSATLSMAYAGAR		
					ANAFVAELK		
A0A452FYF1	Phosphate carrier protein. mitochondrial	92	38067.46	9.53	FGLYEVFK	5.6	Colostrum
					GILSGFGVTLR		
A0A452FZ14	Outer dense fiber protein 1	121	29083.03	8.42	SCGLCDLYPCCLCDVK	17.8	Colostrum
					DVTYSYGLGSCVK		
					LYCLRPSLR		

					CIDELSAR		
A0A452FZ51	Sodium/potassium-transporting ATPase subunit alpha	75	113725.78	6.28	GSDTWFILAR	2807.4	Colostrum
					LGAIVAVTGDGVNDSPALK		
A0A452FZP6	Asparaginase-like protein 1	99	32043.47	6.95	LALFHVEQGK	13.3	Colostrum
					AATVGYNILK		
					GVGGIIMVNK		
					DLSAGAVSAVR		
A0A452G2Q3	Glutamine synthetase	78	42045.47	6.38	RPSANCDPFAVTEALIR	6.7	Colostrum
					YIEEAIEK		
A0A452G3M2	A-kinase anchoring protein 3	203	94838.48	6.52	GTGTAETLLQNAYQAIHNELR	18.4	Colostrum
					SVGEVLQSVLR		
					LLQLSAAAVEK		
					LTNLVIAMAR		
					LYSVIFAK		
					VASELVNETVTACSK		
					ASGTLQSPPNLK		
					NLLSETIFK		
					VDVYSPGDSQPQDWK		
					EVVSDLIDSFMK		
					GFCVDYYNTTSR		

					DTTIATIVLK		
					EGLTLWHNSQQK		
A0A452G4D6	CCT-beta	147	57461.23	6.18	VQDDEVGDGTTSVTVLAAELLR	15.7	Colostrum
					GATQQILDEAER		
					LSSFIGAIAIGDLVK		
					LALVTGGEIASTFDHPELVK		
					DASLMVTNDGATILK		
A0A452G587	Profilin	106	15057.35	8.46	DSPSVWAAVPGK	25.7	Colostrum
					TLVLLMGK		
					TFVNITPAEVGILVGK		
A0A452G667	Leucine-rich repeat-containing protein 37A-like	74	74049.29	4.80	LLSEQQEIK	3.3	Colostrum
					LILSENSLTELHK		
A0A452G800	IF rod domain-containing protein	172	58429.65	7.47	VQQLQTSVDQHGDSLR	24.7	Colostrum
					LLQQQTTTTSVK		
					VDALNDEINFLR		
					NLDLDSIIAEVR		
					AQYEEIAQR		
					FASFIDK		
					GGAGFPVCPAGGIQEVTINQSLLTPLHVEIDPEIQK		

					NLEPFFEAYLNALR		
					LLEGEECR		
					LALDIEIATYR		
A0A452G871	Cytosolic 5'-nucleotidase 1B	107	70113.16	9.07	LINSVNHYGLLIDR	7.9	Colostrum
					YMEYQLTNENVVLTPGPAFR		
					YGTTTAHVPYGISQK		
A0A452G951	Tektin	136	49950.73	5.98	DQAQYGLTDEVHQLEATIAALK	19.8	Colostrum
					EAIALTIAETNNELEAQR		
					NLPLDVAIECLTLR		
					LAQAQDALDALYK		
					FVPEVDTFTR		
					VATEFAFR		
A0A452G9E6	Folate_rec domain-containing protein	166	29761.03	8.30	VLGVPLCK	37.2	Both samples
					TDLLNVCMDAK		
					HFIQDTCLYECSPNLGPWIR		
					AKPGPEDSLHEQCSPWR		
					EDCQIWWEDCR		
					GWNWSAGYNQCPVK		
					FNWDHCGK		
					DISYLYR		
A0A452GAH2	Citrate synthase	125	50583.08	7.33	GLVYETSVLDPDEGIR	8.1	Colostrum

					ALGVLAQLIWSR		
					GYSIPECQK		
A0A452GAV0	Fibronectin type-III domain-containing protein	106	35265.22	5.72	FATVATDLNSFPENNPIQITVQR	16.5	Colostrum
					QQVSFYQVLLQEVVR		
					LMELKPNTSYCLTVR		
A0SWQ7	Kappa-casein	156	15916.54	7.89	SCQDQPTTLAR	40.4	Colostrum
					YIPIQYVLSR		
					HPHPHLSFMAIPPK		
					FFDDK		
					SPAQTLQW		
					VLPNTVPAK		
C7EXB8	Glutathione peroxidase (Fragment)	142	22002.45	9.04	TDVNYTQLVDLHAR	22.6	Colostrum
					ILAFPCNQFGR		
					ICVNGDDAHPLWK		
					FDLFSK		
M4NBE4	78 kDa glucose-regulated protein	193	72397.03	5.07	VEIIANDQGNR	30.2	Both samples
					IINEPTAAAIAYGLDK		
					VTHAVVTVPAYFNDAQR		
					SQIFSTASDNQPTVTIK		

					ITPSYVAFTPEGER		
					TKPYIQVDVGGGQTK		
					LTPEEIER		
					NELESYAYSLK		
					DAGTIAGLNVMR		
					VYEGERPLTK		
					ITITNDQNR		
					ELEEIVQPIISK		
					SDIDEIVLVGGSTR		
					NQLTSNPENTVFDAK		
MANBA	Beta-mannosidase	62	101385.62	6.03	TSAVAPFVWLDVGSIPGR	3	Colostrum
					AVNIIEVR		
A0A097C2Y8	Peptidyl-prolyl cis-trans isomerase (Fragment)	122	23729.50	9.33	VVIGLFGK	17.6	Both samples
					VYFDLR		
					TVDNFVALATGEK		
					IEVEKPFAIAK		
A0A452DMP9	Histone H2B	77	13890.14	10.31	AMGIMNSFVNDIFER	19	Colostrum
					LLLPGELAK		
A0A452DWG4	Ferritin	101	19990.72	6.06	NLNQALLDLHGLASAR	25.1	Colostrum
					QNYSTEVEAAVNR		
					LAGPQAGLGEYLFER		

A0A452DXS2	Testis specific 10	77	68264.90	5.54	VSALADLSSTR	3.9	Colostrum
					LELITAEAEGNR		
A0A452DZA2	Outer dense fiber of sperm tails 2	209	95510.30	7.24	VTDLVNQQQTLEEK	13.3	Colostrum
					LAECQDQLQGYER		
					QFQSQLADLQQLPDILK		
					NIDLTAIISDLR		
					AEVEAIMEQLK		
					LSTFEETNR		
					LEADEVAAQLER		
					QMTCSDINTLTR		
					NYEGMIDNYK		
A0A452EB21	MFS domain-containing protein	82	54149.85	5.77	QVTVLELFR	4.3	Colostrum
					IAESVEMLILGR		
A0A452EC53	Succinyl-CoA:3-ketoacid-coenzyme A transferase	92	54179.78	8.35	AGGAGVPAFYTPTAYGTLVQEGGAPIR	8.2	Colostrum
					YLPDGHIAILSQPR		
A0A452EMJ0	Serine/threonine-protein phosphatase	77	35920.58	6.60	TFTDCFNCLPIAAIVDEK	10.2	Colostrum
					ICGDIHGQYYDLLR		

A0A452EP39	Serine/threonine-protein phosphatase	71	76585.90	5.74	ILQDIASGSHPFSQVLQEAK	5.1	Colostrum
					FASEIAGVDDLGTTGR		
A0A452F333	Cadherin-1	60	98224.21	4.78	VSFEGCAGLPR	2.3	Colostrum
					TVYVSDDTR		
A0A452F9Q0	Tubulin alpha chain	255	50282.78	4.91	IHFPLATYAPVISAEK	43.6	Colostrum
					NLDIERPTYTNLNR		
					FDGALNVDLTEFQTNLVPYPR		
					AVCMLSNTTAIAEAWAR		
					TIQFVDWCPTGFK		
					LIGQIVSSITASLR		
					AYHEQLSVAEITNACFEPANQMVK		
					DVNAAIATIK		
					TIGGGDDSFNTFFSETGAGK		
					AVFVDLEPTVIDEVR		
					ELIDLVLDR		
					EIVDLVLDR		
					AVFVDLEPTVVDEVR		
A0A452G0J3	Metalloproteinase inhibitor 2	73	24360.20	7.44	FFACIK	6.8	Colostrum
					SDGSCAWYR		
A0A452G7E7	SERPIN domain-containing protein	74	46056.87	7.72	IAQLPLTGSTSIIFFLPQK	9.9	Colostrum

					IGFEWNEDGAGTNSSPGVQPAR		
A0A452DVG8	Peptidyl-prolyl cis-trans isomerase (Fragment)	122	48141.37	4.95	TGEAIVDAALSALR	12.5	Mature
					GSTAPVGGGAFPTISTR		
					NSYLEVLLK		
					LAAVDATVNQVLASR		
A0A452E2H7	Elongation factor 1-alpha	97	50140.86	9.10	IGGIGTVPVGR	4.1	Mature
					LPLQDVYK		
A0A452F6V7	Adipsin	110	27911.10	8.09	LQHLLLPVLDR	21.2	Mature
					AVLGPAVQLLPWQR		
					VVPHPGSRPETIDHDLLLLQLSEK		
					LYDVLR		
A0A452FUQ2	Perilipin	120	47594.77	6.36	GAVQSGVDLTK	5.7	Mature
					SMVASGVHSVVGSR		
A0A452G7V5	Protein disulfide-isomerase	190	63093.20	5.12	VDATEESDLAQQYGVR	18.4	Mature
					THILLFLPK		
					VHSFPTLK		
					YKPESDELTAEK		
					ILEFFGLK		
					LITLEEEMTK		
					LLDFIK		

					NFEEVAFDEK		
					ALAPEYAK		
					IFGGEIK		
					EADDIVNWLK		
Q6S4N9	Fatty acid binding protein 3	161	14761.86	6.11	LILTLTHGSAVCTR	39.1	Mature
					SLGVGFATR		
					LGVEFDETTADDR		
					SIVTLDGGK		
					NTEISFK		
A0A452EIE6	Perilipin	185	41306.07	8.14	DAVTTTVTGAK	30.7	Mature
					DSVTSTITGVVDR		
					LPILNQPTNQVVANAK		
					AYQQALCR		
					LEPQIAVANTYACK		
					TITSVAVTSALPIIQK		
					SVVSGSINTVLGSR		
					EVSDGLLASSK		
					SELLVDQYLPLTK		
A0A452F4D1	Odorant-binding protein 4	97	19460.06	4.65	GTSFTQEEFQK	11	Mature
					IVEGGPLR		
A0A452E1G9	HATPase_c domain-	117	92467.84	4.76	SILFVPTSAPR	5.1	Mature

	containing protein						
					FAFQAEVNR		
					ELISNASDALDK		
					SGYLLPDTK		
A0A452E633	ADP ribosylation factor 1	82	20696.77	6.31	DAVLLVFANK	8.8	Mature
					TTILYK		
A0A452ECE5	Endoplasmic reticulum resident protein 29	84	28876.19	5.63	SLNILTAFQK	8.5	Mature
					GALPLDTITFYK		
A0A452F2C6	FERM domain-containing protein	73	68787.27	6.06	IGFPWSEIR	3.3	Mature
					APDFVFYAPR		
A0A452F2R2	GLOBIN domain-containing protein	114	16604.08	6.79	VVAGVANALAHR	25.3	Mature
					AAVTGFWGK		
					LLVVYPWTQR		
A0A452F4U3	3-hydroxyacyl-[acyl-carrier-protein] dehydratase	112	298571.69	6.75	GYAVLGSQEGSQEVQQVPTSK	1088.9	Mature

					TGTVLLEVR		
A0A452FKN8	Transketolase_1 domain-containing protein	117	67890.75	7.20	TVPFCSTFAAFFTR	6.6	Mature
					LAVSQVPR		
					ILATPPQEDAPSVDITNIR		
A0A452FUH2	IF rod domain-containing protein	80	49052.99	5.60	AQYDELAQK	4.1	Mature
					IVLQIDNAR		
A0A452G8Q3	ATP-binding cassette sub-family G member 2	94	71723.43	8.64	SSLLDILAAR	4.2	Mature
					TIIFSIHQPR		
					VGTQFIR		
D3K382	Protein disulfide-isomerase	127	57015.70	6.23	YGVSGYPTLK	7.9	Mature
					DPNIVIAK		
					GFPTIYFSPANK		
					ELSDFISYLK		
A0A452EGM8	Vesicle amine transport 1	75.14	42751.41	6.03	VVTYGMANLLTGPK	6.71	Both samples
					LLALYNQGHIKPR		

a. theoretical value

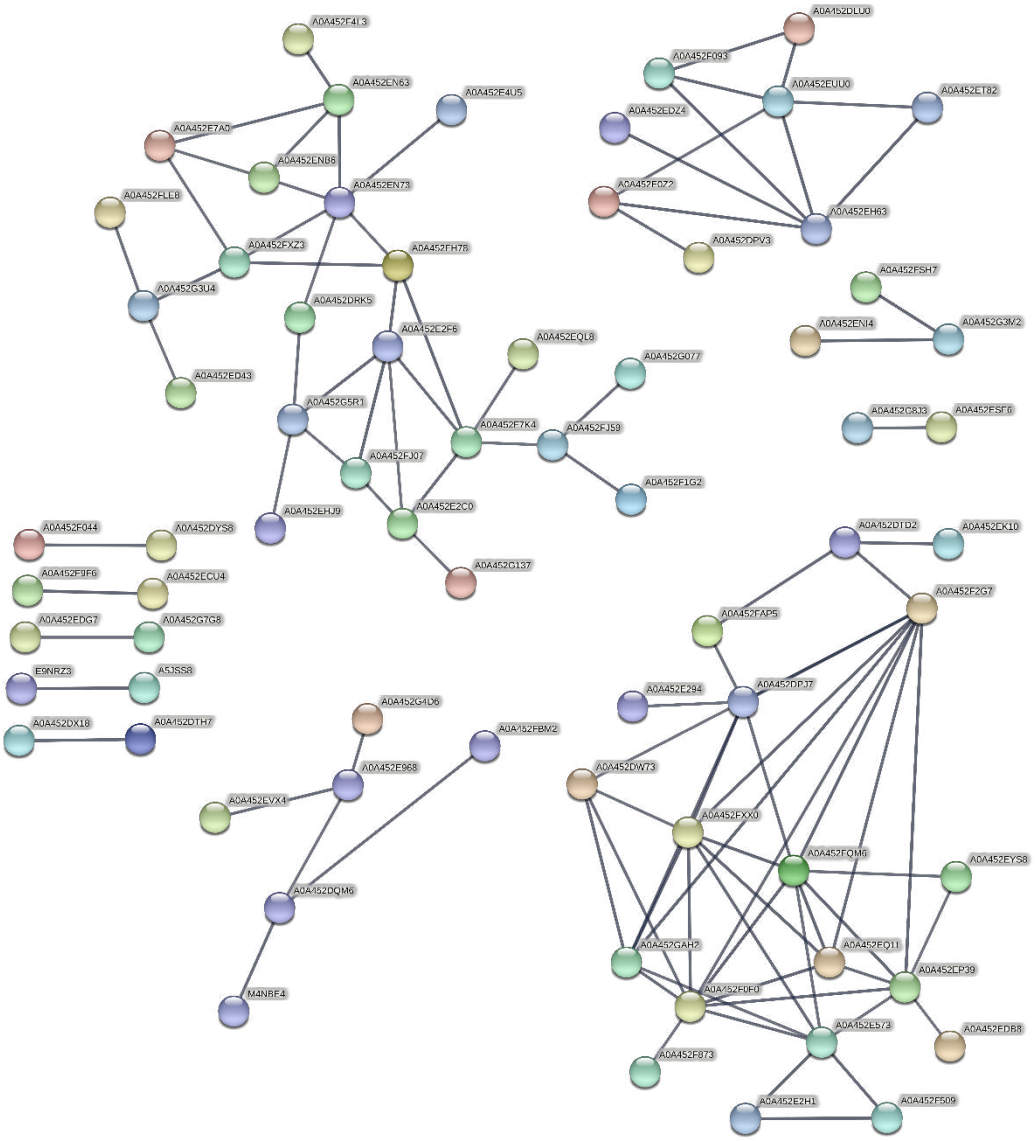
Supplementary table 3. Differentially abundant proteins in goat milk, in colostrum and mature, where "up" corresponds to "upregulated protein". Source: Silva, 2022. Authorial.

ID	Protein name	MW (kDa)[a]	pI[a]	Colostrum	Mature
A0A452EGM8	Vesicle amine transport 1	42751.41	6.03	up	
A0A452F7K4	Albumin	69173.26	5.80	up	
A0A452E2C0	Transthyretin	13633.36	5.58	up	
A0A452DZD6	Ig-like domain-containing protein	11298.58	8.80	up	
A0A452E8D3	Ig-like domain-containing protein	14523.15	8.93	up	
A0A452EPC6	Immunoglobulin mu heavy chain	52990.67	6.50	up	
A0A452G137	Gc-globulin	52366.90	5.41	up	
A0A452EEM4	Peptidoglycan-recognition protein	21050.07	9.37	up	
A0A452F4L3	Fibrinogen alpha chain	66999.17	6.52	up	
A0A452EN63	Fibrinogen beta chain	54468.40	7.11	up	
A0A452EN73	Fibrinogen gamma chain	50405.32	5.78	up	
A0A452E2Y0	Clusterin	50892.66	5.77	up	
A0A452G9T9	Alpha-1-B glycoprotein	53563.51	5.45	up	
A0A452DX18	Complement C3	187307.46	6.07	up	
A5JSS8	Alpha-lactalbumin	16254.61	5.06		up
A0A452E2P1	Immunoglobulin alpha heavy chain	34884.60	8.38		up
A0A452EH90	Lactadherin	47866.36	7.04		up

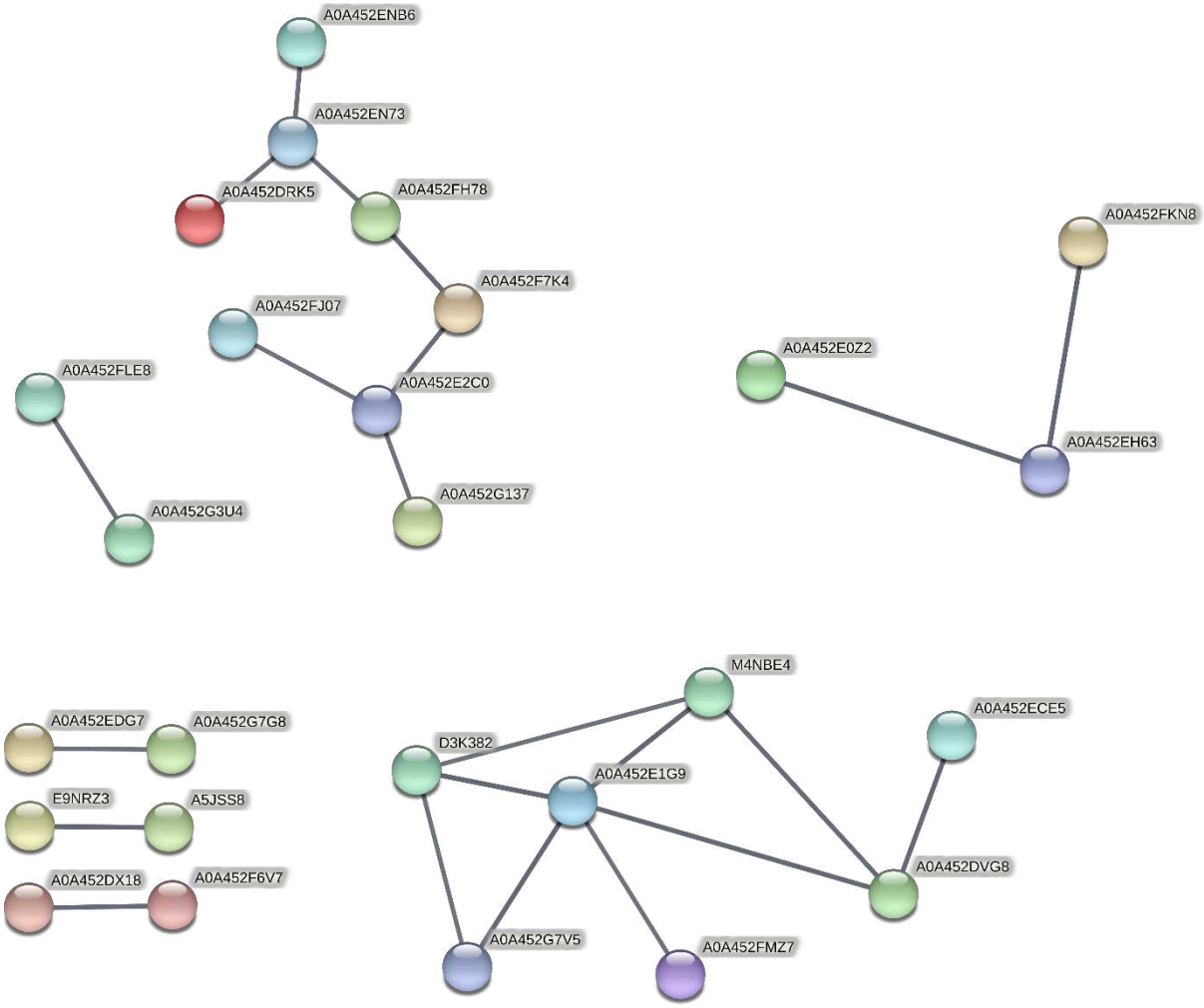
P81447	Glycosylation-dependent cell adhesion molecule 1	17055.54	5.27	up
M4NBE4	78 kDa glucose-regulated protein	72397.03	5.07	up
A0A452EZ30	Leucine rich alpha-2-glycoprotein 1	39058.05	6.76	up
Q6S4N9	Fatty acid binding protein 3	14761.86	6.11	up
A0A452EIE6	Perilipin	41306.07	8.14	up

a. theoretical value

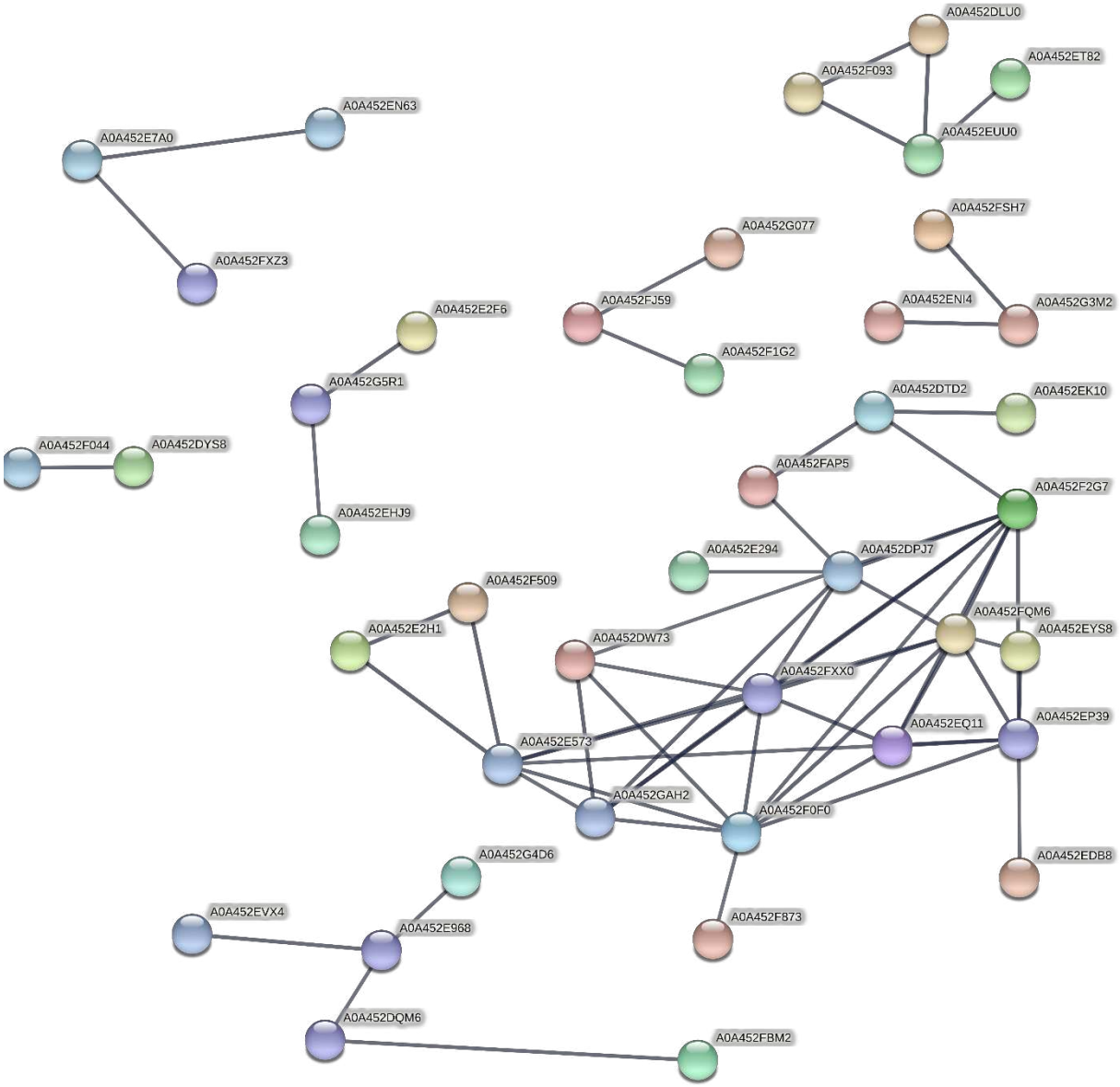
Supplementary figure 1. Interaction network of the proteins identified exclusively in colostrum milk and the common proteins between the two samples. Source: Silva, 2022. Authorial.



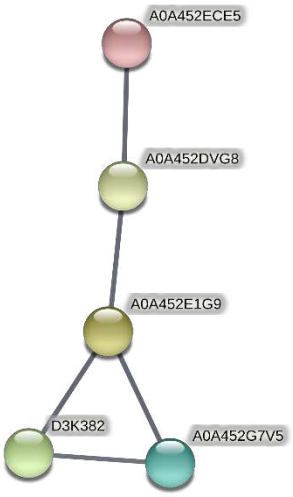
Supplementary figure 2: Interaction network of the proteins identified exclusively in mature milk and the common proteins between the two samples. Source: Silva, 2022. Authorial.



Supplementary figure 3: Interaction network of the proteins identified exclusively in colostrum milk. Source: Silva, 2022. Authorial.



Supplementary figure 4: Interaction network of the proteins identified exclusively in mature milk. Source: Silva, 2022. Authorial.



Supplementary table 4: Protein determination in colostrum and mature milk determined by Bradford´s method. Source: Silva, 2022. Authorial.

Milk sample	Medium
Colostrum	0.003
Mature milk	0.058