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Whey protein dynamics in goat mammary secretions during colostrum and early lactation periods

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Research Article

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Abstract

The protein composition in goat milk undergoes changes throughout the different lactation periods, displaying distinct characteristics that are influenced by the dynamic nature of protein composition and concentration during the transition from colostrum secretion to mature milk. To evaluate the dynamics of whey proteins of Saanen goats during the colostral phase and the first month of lactation, 110 milk samples from 11 healthy mammary halves of seven Saanen goats were selected through a clinical evaluation. Whey was obtained by rennet coagulation of the mammary secretion. The biuret method determined total protein concentration, and their fractions were identified by 12% dodecyl sulfate-polyacrylamide gel electrophoresis. Maximum concentrations of all protein fractions were observed in the first 12 h of lactation, reducing throughout the study. Modification of the protein predominance was also observed. The transition from colostrum secretion to milk occurred 5 or 7 d postpartum.

Extensive research has been conducted on the nutritional and functional properties of milk proteins. Recently, proteomic techniques have been employed to extend knowledge of minor proteins, including those in low abundance. Specifically, various studies have focused on the whey proteome of goat milk (Sun *et al.*, 2020). Investigations into the whey proteomes at different stages of lactation have been carried out in both bovine (Yang *et al.*, 2017; Raimondo *et al.*, 2018) and goat species (Raimondo *et al.*, 2013a; Sun *et al.*, 2020). Previous studies have also examined goat milk whey proteomes at different lactation stages (Raimondo *et al.*, 2013b; Hernández-Castellano *et al.*, 2014). A recent study in the dairy industry focused on the evaluation of whey proteins from goat colostrum and mature milk using proteomic techniques (Sun *et al.*, 2020). The same researchers evaluated the dynamics of whey protein concentration during the colostral and early lactation periods in cows, utilizing the polyacrylamide gel electrophoresis (SDS-PAGE) technique (Raimondo *et al.*, 2018). We wished to extend this to approach to goat milk, therefore, the research described here aimed to investigate the whey protein concentration in goat mammary secretions during the colostrum and early lactation periods using the SDS-PAGE technique.

Material and methods

110 samples from 11 healthy mammary halves of seven Saanen goats were obtained over 10 occasions within the first 30 d in milk (DIM), namely at 0–12 h, 12–24 h and then at 2, 3, 5, 7, 10, 15, 20 and 30 DIM. The animals were kept in pens with access to sunlight, fed hay twice a day and received concentrates containing 14% crude protein in the morning. They were machine milked twice daily.

Firstly, the samples were submitted for microbiological examination. Three positive mammary halves on microbiological analysis were withdrawn from the study. The total protein was determined by infrared radiation. Whey was obtained from milk coagulation, and the determination of its protein was carried out through the Biureto method adopted by Raimondo *et al.* (2010). The fractionation of whey proteins was determined by 12% polyacrylamide gel electrophoresis (SDS PAGE) according to Raimondo *et al.* (2013b). An example electropherogram is given at Supplementary Figure S1.

A descriptive analysis of data was performed and mean, standard deviation, and respective intervals with 95% confidence were obtained. The percentage of each fraction related to whey protein was calculated to describe protein predominance. For the inferential analysis, we first confirmed the hypothesis that the data followed a normal distribution with Kolmogorov–Smirnov test. The measured data were analyzed taking into account the goat and the sampling time during early lactation period applying an ANOVA design with repeated measurements ($P \leq 0.05$) by using the following model: Yij = + *i* + *j* + eij (*i* = 1,…, 40; *j* = 1,…, 7) in which = mean of all data; *i* = is the effect of the *i*th goat; *j* = is the effect of the *j*th moments during early lactation period (group); eij = represents the residual between quarters error. The means were compared with paired Student's *t*-test testing the paired differences between them. Casein was calculated as the difference between total protein content and whey protein content.

Results and discussion

The high concentration of whey protein in colostrum (Table 1), which peaked in the first 12 h of lactation, as well as the marked decrease of this concentration over the days observed in the present research, are in accordance with the reports of previous studies regardless of the technique used (Sanchez-Macias *et al*., 2014; Marziali *et al*., 2018).

The concentration of whey protein and the obtained fractions (Table 1) were also high 12 h postpartum. They decreased abruptly between 12 and 24 h of lactation and continued to decrease steadily until the end of the study period. This steady decline was different from what was observed in cows, where there was stability in whey protein concentration starting from the third day of lactation (Raimondo *et al*., 2018). Using the same SDS-PAGE technique, these authors demonstrated that the transition from colostrum to milk began between 24 and 72 h postpartum in cows. We now show that the dynamics of whey proteins in goat milk during the physiological adaptation of the mammary gland for the transition from colostrum secretion to milk occur later compared to cows.

In the fractionation of the proteins by 12% SDS-PAGE during the colostral phase and the first month of lactation of Saanen goats, the following protein fractions were identified and quantified: lactoferrin (84.0 ± 4.0 kDa); albumin (66.0 ± 2.0 kDa); heavy chain immunoglobulin (52.0 ± 2.0 kDa); light chain immunoglobulin (26.0 ± 1.5 kDa); β -lactoglobulin (β-LG) (16.0 ± 1.0 kDa) and α-lactalbumin (α-LA) (12.0 ± 0.65 kDa). Identifying whey proteins through 12% SDS-PAGE using purified proteins has already been described as efficient in previous studies (Raimondo *et al*., 2013a, 2013b, 2018).

The colostral phase strongly influenced the dynamics of goat milk proteins. In percentages (Fig. 1), within the first 24 h of lactation, whey protein represented over 80% of the total protein content, declining to approximately 30% by the 5th day. During full lactation, goat milk exhibited an approximate protein ratio 20:80 for whey proteins and casein (Selvaggi *et al*., 2014). Regarding the obtained fractions, immunoglobulins prevailed in the early lactation hours (41% within the first 12 h and 36% on the 1st day). In contrast, the concentration of the major whey proteins, β-LG (12 h: 26%; 24 h: 27%) and α-LA (12 h: 9%; 24 h: 12%) accounted for 35% within the first 12 h and 39% on the 1st day of lactation. Chen *et al*. (1998) observed that in the first colostrum collected from goats, approximately 50% of the total proteins consisted of immunoglobulins. This proportion decreased to 30% of the total protein by the end of the first day. In cows, immunoglobulins were the predominant protein, comprising 48% in the first 12 h and 47% between 12 and 24 h of lactation in the total whey protein fraction (Raimondo *et al*., 2018). These authors noted that on the 3rd day of lactation, immunoglobulins accounted for 37%, while the major whey proteins β-LG and α-LA began to predominate, representing 47% of the total whey protein.

On the 5th day of lactation, the protein profile underwent a shift, with the significant whey protein fractions, β-LG (30%) and α-LA (21%), accounting for a combined 51%, while immunoglobulins represented 29%. This dominance was maintained until the end of the study, where β-LG and α-LA remained as the predominant fractions, comprising 57%, while immunoglobulins contributed 23%. In this study, the transition of goat colostrum to milk was considered complete after 5 or 7 d, as all measured parameters fell within the normal ranges reported in the literature for goat milk (Supplementary Figure S2). From day 1 to day 5, the secretion can be classified as transitional goat milk (Sanchez-Macías *et al*., 2014).

Colostrum is mainly characterized by its very high level of IgG, essentially of the IgG_1 subclass, which is actively passed from the serum to the mammary gland during the last weeks before parturition with the function of contributing to neonatal passive immunity (Levieux *et al*., 2002). Due to their importance, immunoglobulins are the most studied colostrum proteins through several evaluation methods. Regardless of the method used, there is a consensus about the maximum concentration of immunoglobulin in colostrum and its decrease with the advancement of lactation (Table 1), which was also observed in the present research and a previous study performed on colostrum from cows (Raimondo *et al*., 2018).

The concentrations of β-LG were higher than those of α-LA throughout the entire period, and their behavior differed. The levels of β-LG decreased abruptly in the first 48 h of lactation, while the decrease in α-LA was slower and more gradual. Others have observed that the concentrations of α-LA and β-LG remain stable in goats during the first five days of lactation and increase as the levels of immunoglobulins decrease (Chen *et al*., 1998). These major whey proteins, α-LA and β-LG, are crucial in determining the nutritional value and functional properties of whey and whey products.

The importance of the increased content of β-LG relates to the fact that it is a significant protein in the whey of ruminant milk and may play important functions in the binding and transport of hydrophobic ligands such as retinoids, alkenes, and fatty acids in addition to being an important source of amino acids for the offspring (Kontopidis *et al*., 2004). α-LA is a small, acidic-cation binding milk protein, which is very important from several points of view being an essential enzyme component for lactose synthesis, a model Ca2+ binding protein, classic molten globule and possessing important biological and functional properties (Stănciuc and Râpeanu, 2010).

Following the same pattern as the other fractions, the concentrations of albumin and lactoferrin (Table 1) were also influenced by the colostral phase. They reached their maximum concentrations within the first 12 h and underwent a sharp decrease on the 1st day of lactation. These proteins play an essential role in the immunity of the mammary gland, and the increase in their concentration coincides with the higher susceptibility to intramammary infections. It may signify an organic reaction that precedes disease, considering that the evaluated glands in the present study were healthy.

According to Shamay *et al*. (2005), albumin synthesis occurs in mammary tissue, and situations involving inflammatory processes, such as mastitis and the dry period, could substantially increase its secretion by the mammary gland. The elevation of albumin levels in milk reflects udder inflammation during the postpartum period and an increase in vascular permeability (Zhang *et al*., 2011). In similar fashion, lactoferrin plays a

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Table 1. Total milk protein, whey protein and various whey protein fractions in goat mammary secretions during the first month of lactation determined by SDS PAGE

		0–12 h	12–24 h	2nd day	3rd day	5th day	7th day	10th day	15th day	20th day	30th day
Total protein (g/dl)	Mean	7.12^{a}	4.25^{b}	4.12^{b}	4.0^{b}	3.79^{b}	3.45^{c}	3.1^{c}	3.02^{c}	2.98^{c}	2.83^{c}
	SEM	(1.3)	(0.7)	(0.8)	(0.5)	(0.6)	(0.4)	(0.3)	(0.2)	(0.1)	(0.2)
Whey protein (mg/dl)	Mean	6033.0^{a}	3342.0^{b}	1973.0^{cd}	1555.0^{d}	1269.0^{c}	1162.0^{ce}	1145.0^{ce}	805.0^{e}	850.0^{e}	856.0^{e}
	SEM	(2773.0)	(2100.0)	(1161.0)	(505.0)	(352.0)	(318.0)	(353.0)	(119.0)	(192.0)	(178.0)
MW (244–132 kDa) (mg/dl)	Mean	141.0^{a}	68.0^{b}	nd	nd	nd	nd	nd	nd	nd	nd
	SEM	(70.0)	(92.0)								
Lactoferrin (mg/dl)	Mean	418.0^{a}	180.0^{b}	133.0^{b}	127.0^{b}	112.0^{b}	91.0^{b}	95.0^{b}	63.0^{b}	6.03^{b}	73.0^{b}
	SEM	(277.0)	(107.0)	(80.0)	(52.0)	(34.0)	(29.0)	(56.0)	(16.0)	(30.0)	(34.0)
Albumin (mg/dl)	Mean	455.0^{a}	202.0^{b}	143.0^{b}	131.0^{b}	127.0b	108.0^{b}	137.0^{b}	104.0^{b}	104.0^{b}	98.0^{b}
	SEM	(259.0)	(150.0)	(82.0)	(51.0)	(41.0)	(29.0)	(78.0)	(21.0)	(30.0)	(29.0)
Heavy chain Ig (mg/dl)	Mean	1142.0^{a}	576.0^{b}	303.0^{c}	242.0^{c}	183.0^{c}	149.0^{c}	160.0^{c}	103.0^{c}	113.0^{c}	102.0c
	SEM	(426.0)	(471.0)	(315.0)	(187.0)	(107.0)	(92.0)	(105.0)	(28.0)	(48.0)	(32.0)
MW (30–50 kDa) (mg/dl)	Mean	324.0^{a}	331.0^{a}	244.0^{a}	95.0^{b}	17.0^{b}	72.0^{b}	18.0^{b}	32.0^{b}	nd	nd
	SEM	(353.0)	(171.0)	(125.0)	(104.0)	(30.0)	(75.0)	(42.0)	(34.0)		
Light chain Ig (mg/dl)	Mean	1431.0^{a}	736.0^{b}	327.0^{c}	258.0^{c}	194.0^{c}	176.0^{c}	173.0^{c}	62.0^{c}	100.0^{c}	99.0^{c}
	SEM	(879.0)	(678.0)	(307.0)	(150.0)	(117.0)	(80.0)	(113.0)	(28.0)	(38.0)	(29.0)
Beta-lactoglobulin (mg/dl)	Mean	1593.0^{a}	888.0^{b}	553.0^{c}	443.0^{c}	379.0^{c}	338.0^{c}	350.0^{c}	292.0^{c}	306.0^{c}	323.0^{c}
	SEM	(909.0)	(598.0)	(314.0)	(143.0)	(87.0)	(86.0)	(75.0)	(45.0)	(54.0)	(48.0)
Alpha-lactalbumin (mg/dl)	Mean	527.0^{a}	359.0^{b}	266.0^{b}	256.0^{b}	255.0^{b}	226.0^{c}	211.0^{c}	147.0^{c}	162.0^{c}	160.0^{c}
	SEM	(168.0)	(123.0)	(95.0)	(65.0)	(55.0)	(31.0)	(47.0)	(31.0)	(27.0)	(31.0)

Values are mean and SEM.
Different letters on the same line mean $P \leqslant 0.05$, Students *t* test. nd, nondetectable; MW, molecular weight

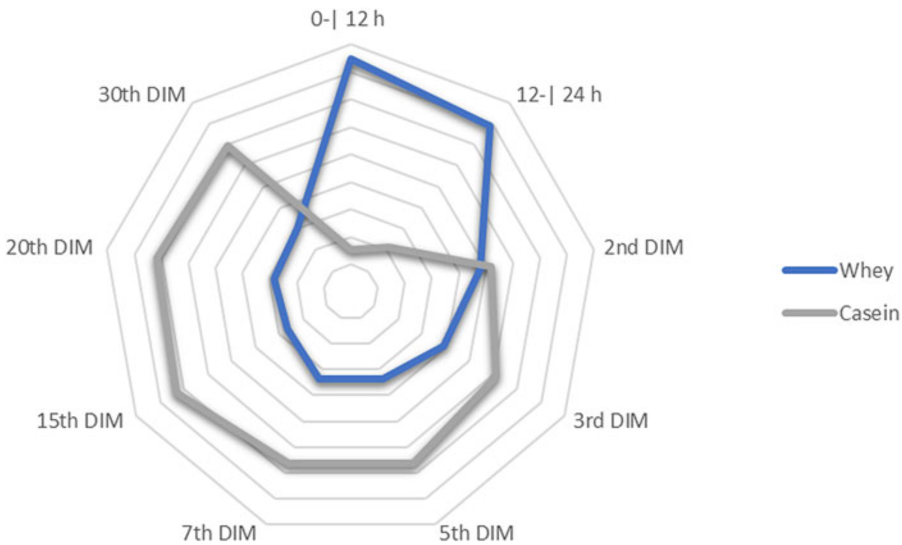

Figure 1. Radar graph showing the percentage of whey protein and casein (obtained by difference between total protein and whey protein) in the total milk protein of goats during the first month of lactation.

crucial role in the defense of the mammary gland (McGrath *et al.*, 2016). Its high concentration during the first days of lactation may be related to a possible function in preventing infections.

In addition to the commonly observed whey proteins in goat milk, unidentified protein fractions were detected and presented based on their molecular weights. These protein fractions appeared before the lactoferrin fraction, with molecular weights ranging from 132 to 244 kDa and between the heavy chain immunoglobulins and light chain immunoglobulins, 29 to 43 kDa. These same protein fractions were observed in a previous study involving cow colostrum (Raimondo *et al.*, 2018). The authors attributed this finding to the increased blood flow in the mammary gland during the preparatory phase of parturition, resulting in physiological inflammation and increased vascular permeability, allowing serum proteins to pass into the mammary gland.

The behavior of these unidentified protein fractions was like the other whey proteins (Table 1). The fraction with a molecular weight between 132 and 244 kDa exhibited the highest average levels within the first 12 h of lactation and decreased abruptly in samples collected at 24 h, no longer being observed after that. No studies were found in the reviewed literature that mentioned the presence of these fractions, except for the survey conducted on cow colostrum by the same researchers (Raimondo *et al.*, 2018).

The proteins with a molecular weight between 30 and 50 kDa showed peak concentrations within the first 12 h of lactation (Table 1). These levels declined rapidly within five days, stabilized on the 15th day of lactation, and were no longer observed in samples collected from 20 d onwards.

In conclusion, we have confirmed that the whey protein fractions in goat milk exhibit maximum concentrations within the first 12 h of lactation, followed by a gradual decrease over the first month. This pattern reflects the nutritional and immunological quality of the colostral secretion in goats. The transition from colostrum to milk in goats is typically achieved after 5 or 7 d, as indicated by all measured parameters falling within the normal ranges described in the existing literature for goat milk. The percentage distribution of protein fractions plays a significant role in shaping whey protein profile. A predominant presence of immunoglobulins characterizes colostrum, while β-LG and α-LA become the predominant fractions from the 2nd day onwards.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S002202992400013X

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