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The dynamics of individual whey protein concentrations in cows' mammary secretions during the colostral and early lactation periods

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Research Article

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Abstract

The bovine whey consists of more than 200 different types of proteins, of which β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins and lactoferrin predominate. However, their concentrations are not stable due to the existence of protein dynamics during a transition from colostrum secretion to mature milk. To evaluate the dynamics of whey proteins of Jersey cows during a colostral phase and first month of lactation and an influence of the number of lactations, 268 milk samples from 135 Jersey cows were selected through a clinical evaluation. Whey was obtained by rennet coagulation of the mammary secretion. The concentration of total proteins was determined by the biuret method and their fractions were identified by 12% dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE). Maximum concentrations of all protein fractions were observed in the first 12 h of lactation, reducing over the course of the study. Modification of the protein predominance was also observed. The transition from colostrum secretion to milk occurred between 24 and 72 h postpartum. There was an influence of the number of lactations on the dynamics of whey proteins, indicating that multiparous cows had better immunological and nutritional quality when compared to primiparous cows.

The first month of lactation is characterized by the secretion of colostrum and the transition of this secretion into milk. The high amount of whey proteins in colostrum is due not only to immunoglobulins, but also to other proteins that promote the development and maturation of the epithelial tissues of the neonatal gastrointestinal system (Guilloteau et al. 2009).

The research carried out by Hillier (1976) showed that the technique of polyacrylamide gel electrophoresis in bovine whey was adequate for the isolation and identification of milk proteins and enzymes. The technique was recommended for detection of proteins, even those that would occur in small concentrations in whey (Raimondo et al. 2013*b*). This technique allows evaluation of the whey protein dynamics, providing knowledge of possible factors of variability in the protein composition of the milk. In the analyzed literature, no studies were found evaluating the protein fractions of whey during the initial phase of lactation through the technique of polyacrylamide gel electrophoresis – the research was dedicated to the study of one or more fractions, especially the immunoglobulin. Therefore, the aim of this research was to study the dynamics of whey proteins concentration in cows' mammary secretion during colostral and early lactation periods and the possible influence of the number of lactations on whey proteins during the first 72 h of lactation.

Material and methods

Herd and cow selection

The animals used were from semi-extensive breeding systems located in the Brazilian state of São Paulo. Food was based on roughage, supplemented with silage and commercial concentrate and machine milking system were used. The inclusion criteria for cows were based on the clinical examination and history, with exclusion of those that had recurrent episodes of mastitis, alterations of the macroscopic characteristics of the milk or alterations in the appearance of the mammary gland, measured by palpation, that could be indicative of previous acute or chronic inflammatory process (Dirksen et al. 1993).

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Experimental design

To study the dynamics of whey proteins in Jersey cows during their early lactation period, 268 samples from 135 Jersey cows were used in eight groups within the first 30 d in milk (DIM): 0–12 h; 12–24 h; 2nd DIM; 3rd DIM; 4th and 5th DIM; 6th and 7th DIM; 8th to 15th DIM, and 16th to 30th DIM.

To evaluate the influence of the number of lactations on whey proteins during the first 72 h of lactation, 117 samples comprising 50 from 28 primiparous cows and 67 from 32 multiparous cows, were divided into three groups: (a) 0–24 h (18 primiparous and 29 multiparous cows); (b) 24–48 h (16 primiparous and 19 multiparous cows) and (c) 48–72 h (16 primiparous and 19 multiparous cows).

Sample collection and laboratory analysis

Three aliquots of milk from each quarter were collected in the milking parlor prior to milking: an aliquot for microbiological examination in sterile plastic tubes, an aliquot for somatic cell count (SCC) and total protein (40 mL into plastic flasks with 8 mg of bronopol as a preservative) and an aliquot for obtaining whey (50 mL in plastic bottles kept refrigerated).

The milk samples were submitted to microbiological analysis, seeded in ram's blood-agar medium and incubated at 37 °C, and readings were performed at 24, 48 and 72 h of incubation. All 268 samples used were negative in the microbiological examination. The SCC was executed by Flow cytometry using Somacount 500 (Bentley Instruments Inc.) and total protein was determined by infrared radiation using a Bentley 2000 infrared instrument (Bentley Instruments Inc.).

Whey was obtained from milk coagulation by the addition of 5% renin solution and centrifugation at 16 000 g (13 000 rpm) for 20 m using Biofuge Pico microtube centrifuge from Heraeus Instruments. The turbidity of the sample was eliminated in order to avoid interferences in the measurement of the absorbance in the determination of its protein carried out through the Biureto method adapted by Raimondo et al. (2010*a*), for use in whey through the equation (Whey protein = (Absorbance-0.005)/0.05).

The fractionation of whey proteins was determined by 12% dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Raimondo et al. 2013*b*) with samples prepared in 2-mercaptoethanol sample buffer. The gel was stained with the 0.23% solution of Coomasie Blue R-250 for 90 min., and destained in the methanol/acetic acid solution (5% methanol, 7% acetic acid). Destained gels were scanned with an Epson Expression 1680 Pro scanner. The identification and quantification of proteins by their molecular weight was performed using Life Science Software – Vision Works LS-UVP (Image Acquisitions and Analysis Software) through molecular weight standard, ranging between 250 and 10 kDa (Precision Plus Protein Standards Kaleidoscope Ref. 161–0375 Bio-Rad®) and Lactoferrin (Lactoferrin from bovine milk Ref. L9507), serum albumin (Albumin bovine serum Ref. A7517), bovine IgG (IgG from bovine serum Ref. I9640), β-Lg (β-Lactoglobulin from bovine milk Ref. L4756) and α-La (α-Lactalbumin from bovine milk Ref. L6385) purified proteins by Sigma-Aldrich.

Statistical analysis

Statistical analysis was performed using Graph Pad 6 (Graph Pad Software, San Diego, CA). Data are expressed as mean with standard error. Initially, the data were tested for homogeneity, followed by analysis using ANOVA with Bonferroni post-hoc test (whey proteins and total protein during the first month of lactation), or using the non-parametric Kruskal–Wallis test with a Dunn post-test (SCC). The two-way ANOVA with Bonferroni post-hoc test was performed to evaluate the influence of the number of lactations. $P < 0.05$ was considered statistically significant.

Results and discussion

The high concentration of whey protein in colostrum (Table 1), maximum in the first 12 h of lactation (9049 ± 558.4 mg/dl), 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 (Raimondo et al. 2010*b*; Senda et al. 2011; Zhang et al. 2011). Using the two-dimensional electrophoresis technique, Senda et al. (2011) and Zhang et al. (2011) also observed that the expression of the fractions is maximal in the first hours of lactation and stabilizes from day 3, therefore, we can state that the transition period from colostrum secretion to milk occurs between 24 and 72 h after calving.

The high whey protein concentration follows the high total milk protein concentration (Table 1), which is also maximal in the first 12 h of lactation and decreases over the evaluated period. SCC was maximal in the first week of lactation and decreased over the study period, following the same phenomenon observed in the proteins. The high SCC in colostrum is not attributed to mastitis, but to penetration of cells through the cell junctions opening due to increased vascular permeability (Nguyen & Neville, 1998) and the subsequent decrease shows that the mammary glands are healthy.

In the fractionation of the proteins during the colostral phase and the first month of lactation of Jersey cows by 12% SDS-PAGE, the following protein fractions were identified and quantified (Fig. 1): 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); B-lactoglobulin (β-LG) (16.0 ± 1.0 kDa) and α-lactalbumin (α-LA) (12.0 ± 0.65 kDa). The identification of whey proteins through 12% SDS-PAGE using purified proteins has already been described as efficient in previous studies (Raimondo et al. 2013a; 2013*b*).

On the first day of lactation, the predominant protein was immunoglobulin, corresponding to 48% in the first 12 h and 47% between 12 and 24 h of lactation of the total whey protein (Fig. 2). In the same period, the main whey fractions, β-LG and α-LA, corresponded to 22% (0–12 h) and 26% (12–24 h). On the 3rd DIM, the immunoglobulins corresponded to 37%, and the whey proteins β-LG and α-LA began to predominate, which together corresponded to 47% of the total whey protein. At the end of the first month of lactation, it was observed that β-LG corresponded to 35% of the total. Together with the 21% represented by α-LA, they corresponded to 56% of whey protein, while during that phase the immunoglobulin corresponded to 28% of the total whey.

Immunoglobulins are the most studied colostrum proteins through several methods of evaluation: radial immunodiffusion (Madsen et al. 2004), two-dimensional electrophoresis (Senda et al. 2011) and ELISA (Chigerwe et al. 2005). 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, which we also observed in the present research (Table 1).

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Table 1. Mean and mean standard deviation (SEM) of the somatic cell count (SCC), total milk protein, whey protein and whey protein fractions, molecular weights (MW) between 240 and 132 kDa; Lactoferrin, Albumin; Heavy Chain Immunoglobulin; Molecular weights between (MW) 30 and 40 kDa; Light Chain Immunoglobulin; β-lactoglobulin and α-lactalbumin (obtained by SDS-PAGE of Jersey cows during the first month of lactation)

		0–12 h	12–24 h	2nd day	3rd day	4th–5th day	6th–7th day	8th–15th day	16th–30th day
SCC (cel/ml)*	Mean	590 100 ab	1 144 000 a	1 033 000 abc	1 191 000 abc	791 600 abcd	640 700 bcd	377 100 cd	173 400 d
	SEM	70 190	353 800	260 700	297 200	283 100	261 000	193 800	75 040
Total protein (g/dl)	Mean	10.6 a	7.14 b	4.86 c	4.57 c	4.28 cd	4.07 de	3.96 de	3.56 e
	SEM	0.30	0.34	0.12	0.09	0.11	0.06	0.14	0.04
Whey protein (mg/dl)	Mean	9049 a	4025 b	1915 c	1525 cd	1256 cd	1163 cd	1148 cd	930.9 d
	SEM	558.4	423.2	150.6	99.54	123.4	53.71	49.56	35.43
MW (240–132 kDa) (mg/dl)	Mean	561.1 a	253.2 b	78.71 c	40.66 cd	27.00 cd	22.89 cd	27.39 cd	15.24 d
	SEM	37.21	30.03	10.59	8.185	6.26	3.905	3.957	2.277
Lactoferrin (mg/dl)	Mean	968.6 a	288.9 b	161.4 c	135.0 c	97.04 c	95.53 c	102.1 c	83.73 c
	SEM	75.80	36.90	16.56	12.27	11.17	6.648	6.103	5.507
Albumin (mg/dl)	Mean	804.5 a	390.8 b	137.4 c	104.6 c	77.88 c	73.95 c	75.78 c	56.63 c
	SEM	68.18	52.45	11.29	10.08	9.623	5.285	6.097	3.709
Heavy Chain Ig (mg/dl)	Mean	1670 a	959.0 b	353.7 c	256.0 cd	182.4 cd	165.7 cd	153.4 cd	105.4 d
	SEM	129.3	116.1	34.50	23.85	29.37	13.66	9.727	7.237
MW (30–40 kDa) (mg/dl)	Mean	498.7 a	138.9 b	96.59 c	62.04 c	22.95 c	24.36 c	14.89 c	13.82 c
	SEM	98.53	33.02	11.45	11.50	7.163	10.46	4.976	6.658
Light Chain Ig (mg/dl)	Mean	2593 a	1025 b	435.5 c	314.0 c	226.4 c	190.2 c	193.1 c	142.5 c
	SEM	188.8	133.0	40.63	27.21	38.39	16.51	14.94	11.66
β-lactoglobulin (mg/dl)	Mean	1600 a	716.7 b	442.8 c	413.5 c	393.3 c	375.8 c	370.2 c	324.8 c
	SEM	130.5	65.81	27.79	20.65	22.16	12.97	16.10	12.97
α-lactoalbumin (mg/dl)	Mean	352.4 a	252.0 b	209.1 c	199.6 bc	228.7 bc	214.6 bc	210.9 bc	188.8 c
	SEM	29.48	12.61	9.691	6.120	11.89	6.060	7066	6.788

Different letters on the same line mean $P \leqslant 0.05$ – Bonferroni test
*Different letters on the same line mean $P \leqslant 0.05$ – Dunn test

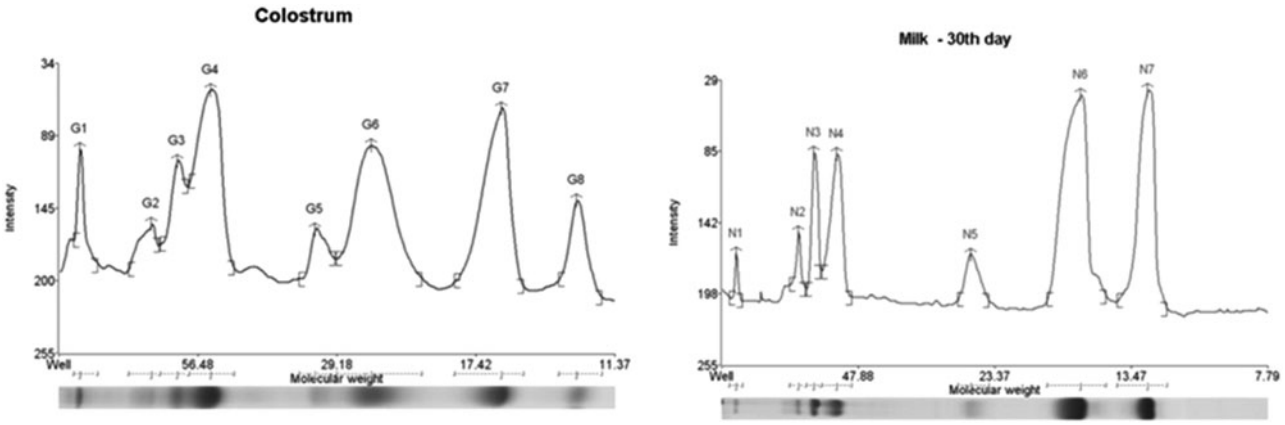

Fig. 1. Electropherogram of the whey proteins of Jersey cows during the colostrum phase and the milk, obtained by dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE). G1, N1 – proteins with molecular weight between 132 and 240 kDa; G2, N2 – lactoferrin; G3, N3 – serum albumin; G4, N4 – heavy chain immunoglobulin; G5– proteins with molecular weight between 30 and 40 kDa; G6, N5 – light chain immunoglobulin; G7, N6 – β-lactoglobulin; G8, N7 – α-lactalbumin.

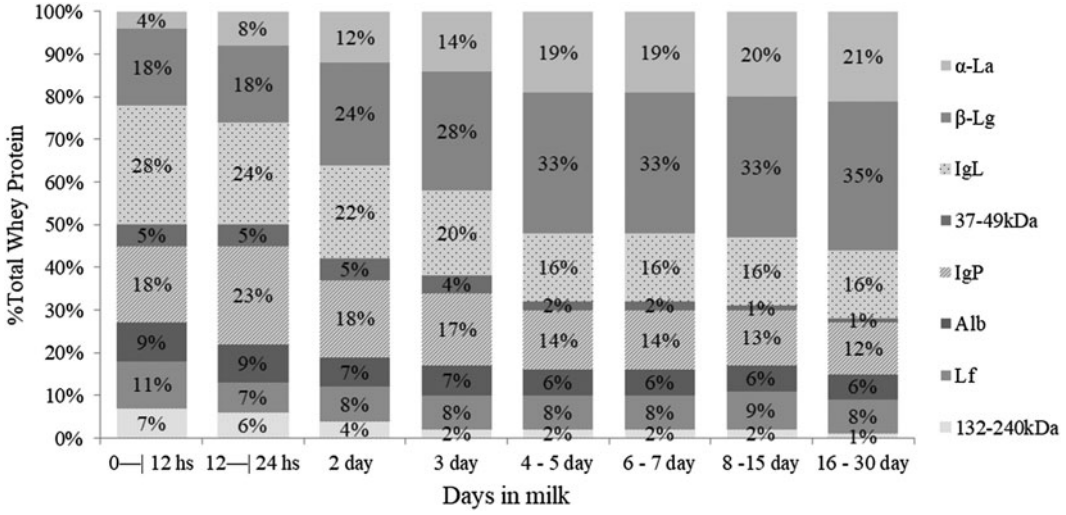

Fig. 2. Concentration (%) of the lactoferrin (Lf), albumin (Alb), heavy chain immunoglobulin (IgP), light chain immunoglobulin (IgL), β-lactoglobulin (B-Lg), α-lactalbumin (α-La).of Jersey cows in relation to the whey protein during the first month of lactation, obtained by dodecyl sulfate-polyacrylamide gel electrophoresis (12% SDS-PAGE).

In contrast to whey proteins, lactose increases over the period after calving to about 120 h, which is confirmed by the increase in the relative amount of α-La in milk, which is a prerequisite for the biosynthesis of lactose (Senda et al. 2011). In the present study, the α-La concentration was maximal in the first 12 h of lactation (Table 1) and it gradually decreased concomitantly with other whey proteins. Despite this decrease, when evaluating the predominance of protein fractions during the first month (Fig. 2), it is observed that α-La represents the smallest fraction in colostrum, while at the end of the study it represents the second most abundant protein.

Albumin and lactoferrin also presented maximum concentrations in the first 12 h after calving (Table 1). The values of albumin abruptly decrease on the 1st day of lactation and from the 3rd day they stabilize, remaining stable until the end of the first month, similar with previous studies (Heng, 1999; Levieux; Ollier, 1999; Zhang et al. 2011). This elevation of albumin in the colostral whey may also be a result of the increase of the blood permeability, since it plays a role in the transport of small molecules (Zhang et al. 2011). Moreover, its level increases with cow's age, and it is likely to be associated with previous inflammations, which resulted in damage to the epithelium that increased its permeability, even after recovery (Króls et al. 2013).

The levels of lactoferrin (Table 1) decreased abruptly during the first two days of lactation, remaining stable for the rest of the 1st month. Lactoferrin is an iron-binding cationic glycoprotein of mammary origin that plays a key role in the defense of the mammary gland by transporting iron to absorption locations and by storage for cells that need iron (Zhang et al. 2011; McGrath et al. 2016). The concentration of lactoferrin in milk is variable, however, several authors have reported an increased colostrum concentration of 30–100 times higher than that of milk (Yoshida et al. 2000; Hiss et al. 2008; Zhang et al. 2011).

During the colostral phase and the first month of lactation, in addition to the proteins normally observed in the whey of cows, other fractions that could not be named were observed and presented by molecular weight. These protein fractions were located before the fraction of lactoferrin, with molecular weights ranging

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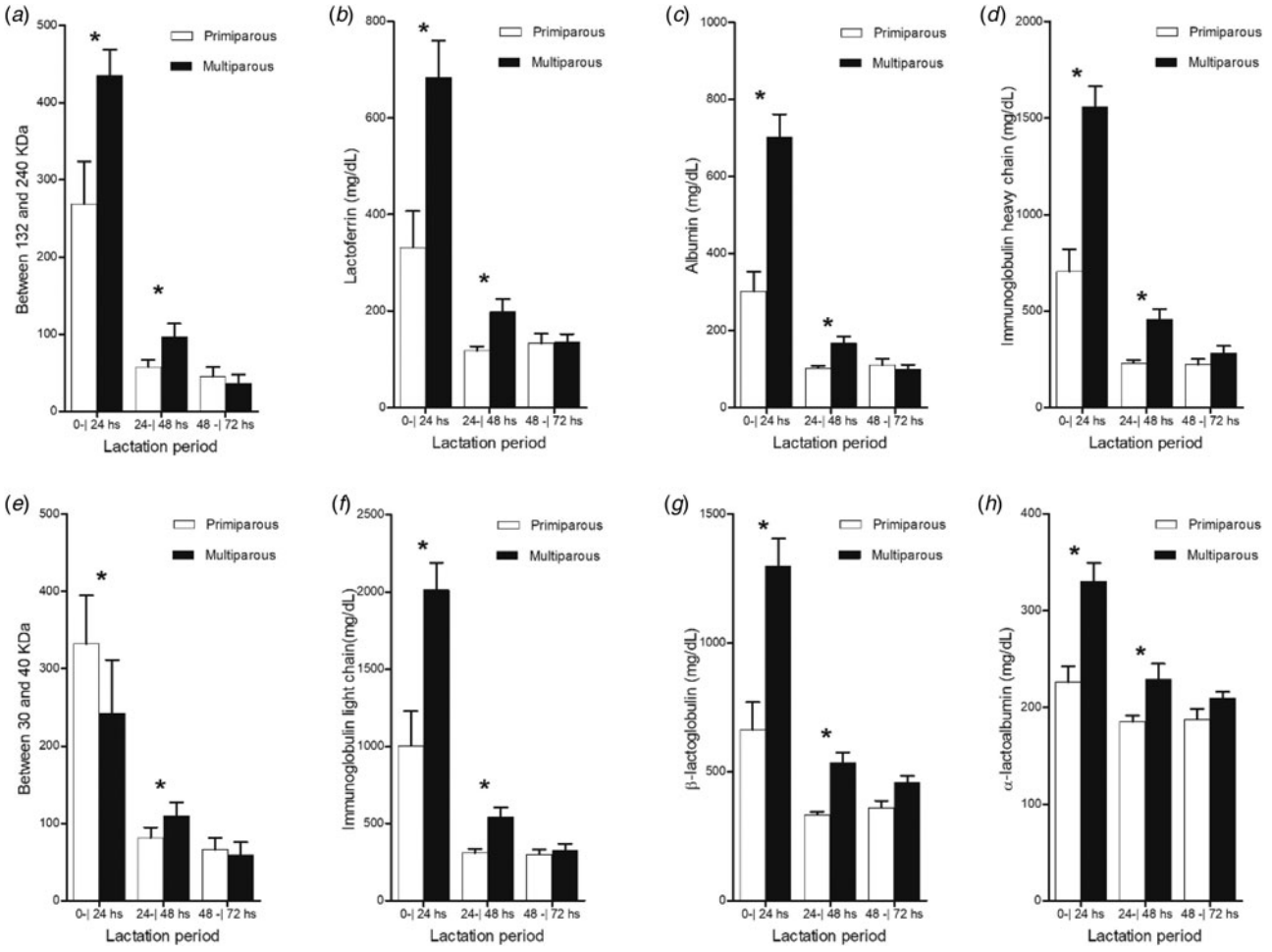

Fig. 3. Electropherogram of the whey proteins, proteins with molecular weight between 132 and 240 kDa, lactoferrin, albumin, heavy chain immunoglobulin, proteins with molecular weight between 30 and 40 kDa, light-chain immunoglobulin, β-lactoglobulin and α-lactalbumin, of primiparous and multiparous Jersey cows during the colostrum. *$P < 0.05$.

from 132 to 240 kDa, and 30 to 40 kDa located between the heavy chain immunoglobulins and light chain immunoglobulins. The presence of these fractions at the beginning of lactation can be explained by the increase in the blood flow that occurs in the mammary gland during the calving preparation phase, causing physiological inflammation and increased vascular permeability with the passage of serum proteins to the mammary gland (Phillippy & Mccarthy, 1979; Zhang et al. 2011).

Whey protein was higher in multiparous cows (7262.0 ± 544.0 mg/dl) during the first 24 h of lactation compared to primiparous cows (3832.7 ± 680.0 mg/dl). Also, the protein concentrations were higher in the cows during the first 48 h of lactation when compared to the concentrations obtained in primiparous cows, except the fraction with molecular weight between 40 and 50 kDa (Fig. 3). These data confirmed what was observed in the total whey protein, demonstrating that not only the immunological quality of the colostrum of primiparous cows is lower, but also the nutritional quality. From the 48 h of lactation, whey proteins are no longer influenced by the number of lactations.

The heavy chain and light chain immunoglobulins were higher in multiparous cows than first lactating cows (Fig. 3). These results support the difference in the immunological quality of the colostrum in primiparous and multiparous cows, confirming the previous reports of Raimondo et al. (2010*b*), who observed a significant difference in total colostrum protein levels of primiparous and multiparous cows.

The concentration of the fraction with molecular weight between 40 and 50 kDa was higher in primiparous cows in the first 24 h, possibly due to the presence of edema and inflammation with increased vascular permeability of the mammary gland. However, there is an inversion of this difference between 24 and 48 h of lactation, when the concentrations in multiparous cows are higher. In addition, the albumin concentrations were higher in cows during the first 48 h of lactation in this study.

The lower concentration of lactoferrin observed in primiparous cows may explain the greater susceptibility of the mammary gland of these animals as observed by Costa et al. (1996), who demonstrated the occurrence of 80% of intra-mammary infections in heifers in the period before calving in Brazilian herds and by Tenhagen et al. (2009) in Germany. Previously, it was observed through simple radial immunodiffusion that multiparous cows had lactoferrin content 2–3 times greater than primiparous cows (Tsuji et al. 1990).

Conclusions

There are changes in whey protein dynamics during early lactation of cows. These changes reflect the transition from colostrum

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secretion to milk, which occurs between 24 and 72 h after calving. Maximum concentrations of all fractions occur at the initial 12 h of lactation and they decrease over the first month, reflecting the nutritional and immunological quality of the colostral secretion. The percentage distribution of the protein fractions modifies the protein profile of the whey with predominant immunoglobulins in the colostrum and β-LG and α-LA predominant from the 3rd day. The colostrum of multiparous cows presented higher nutritional and immunological quality.

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