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Communication

[*Comunicação*]

Influence of parity on concentrations of enzymes, proteins, and minerals in the milk of cows

[*Influência do número de parições nas concentrações de enzimas, proteínas e minerais no leite de vacas*]

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The composition and physical properties of colostrum vary according to a number of factors such as the age of the animal, number of lactation cycles, breed, diet, vaccination, and diseases (Kume and Tanabe, 1993; Tsioulpas *et al*., 2007). For instance, it is well established that first lactation heifers produce significantly less colostrum than multiparous cows and that the quality of this colostrum is usually poor (Petrie *et al*., 1984). Also, dairy cows have more developed mammary glands that often produce a much higher amount of colostrum when compared to beef cows. Colostrum is also the main source of minerals for newborn calves. The concentrations of minerals such as calcium, phosphorus, and magnesium in bovine colostrum are higher at calving and decrease with time (Kume and Tanabe, 1993).

The aim of this study was to evaluate the influence of parity on some parameters of colostrum and milk whey of beef cows in the first 30 days of lactation.

The lacteal secretions of 35 Canchim cows were analyzed. Five groups comprising seven cows each were formed to evaluate whether the number of lactations influenced the content of proteins and the biochemical parameters of colostrum and milk whey. The groups consisted of: seven first lactation cows (Group 1), seven second lactation cows (Group 2), three third and four fourth lactation cows (Group 3), seven fifth lactation cows (Group 4) and seven sixth lactation cows (Group 5). Third and fourth lactation cows were included together in Group 3 because of the small number of animals available for the experiment. The samples of colostrum and milk were taken immediately after calving (day 0) and 1, 2, 7, 15 and 30 days thereafter. All cows were managed under similar conditions prior to and during sample collection. None of the animals used in this study exhibited any health problems during the experimental period. The experimental design was approved by the Ethics Committee on Animal Use at the School of Veterinary Medicine, UNESP at Jaboticabal, under protocol number 009793-08.

Wheys from colostrum and milk were obtained by addition of 5mL of rennet (Coalho Estrella®, Chr. Hansen Brasil Ind. e Com. LTDA, Valinhos, São Paulo, Brazil) to 100mL of lacteal secretions. After stirring and incubation at 37°C for 20 minutes, the whey was separated by centrifugation. Whey samples were then frozen and kept at -20ºC until analysis. Gamma-glutamyltransferase (modified Szasz method), total protein (biuret method), calcium (CPC reaction), phosphorus (modified Daly and Ertinghausen method), and magnesium (Labtest reaction) were then determined spectrophotometrically using commercial reagents (Labtest Diagnostica, Lagoa Santa, Minas Gerais, Brazil). Ionized calcium levels were determined by the ion-selective electrode method (9180 Electrolyte

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Analyzer, Roche Diagnostics, Mannheim, Germany). Whenever necessary, the samples were diluted in deionized water as follows: gamma-glutamyltransferase: 1:150 (day 0), 1:50 (day 1), 1:20 (days 2 to 30); total protein: 1:3 (day 0; no further dilutions in the subsequent time points); calcium, phosphorus, and magnesium: 1:5 (days 0 to 30); ionized calcium: 1:3 (days 0 to 30).

Electrophoretic protein separation was carried out in polyacrylamide gels using the technique described by Laemmli (1970).

The data were submitted to ANOVA and the differences among groups were analyzed with the Tukey's test. Differences were considered significant at $P<0.05$ (Zar, 1999).

Even though gamma-glutamyltransferase (GGT) activity levels did not differ between groups, they were higher in colostrum whey of group 1 as compared to group 3 or later lactation cows (Table 1), which is probably due to concentration of this enzyme in a lower volume of colostrum. There was a decrease in GGT activities during the transition from colostrum to mature milk. Zanker *et al*. (2001) reported a GGT activity of 30,479 IU/L in colostrum whey of primiparous cows two hours after calving and, according to these authors, the activity of this enzyme is very high in colostrum soon after calving and gradually decreases as days go by.

Total protein concentrations were significantly higher in colostral whey of groups 2 and 4 and lower in group 5 (Table 1). Zarcula *et al*. (2010) reported that breed and parity influenced the chemical composition of first colostrum, and that protein content of whole colostrum was higher in cows from second and third lactation compared to those in fourth lactation, differing from the results of the present study.

Calcium concentrations in whey did not differ between groups and gradually decreased until the thirtieth day of lactation (Table 1). Contrary to the results of this study, Kume and Tanabe (1993) reported a higher concentration of calcium in colostrum of primiparous Holstein cows as compared to multiparous cows of the same breed. Ionized calcium concentration was significantly higher in group 2 and lower in groups 3 and 4 (Table 1). Like total calcium levels, ionized calcium concentrations also gradually decreased until 30 days postpartum. In the present study, ionized calcium levels were higher than those reported by Tsioulpas *et al*. (2007).

The inorganic phosphorus content levels of colostrum whey did not differ between groups (Table 1). According to Kume and Tanabe (1993), inorganic phosphorus concentrations are higher in colostrum of primiparous Holstein cows as compared to multiparous cows and remain constant after the third lactation. These results differ from the findings of the present study most likely because colostrum whey instead of whole colostrum was analyzed.

The concentrations of calcium and inorganic phosphorus in cow colostrum described in literature are 3-4 times higher than those found in the present study (Tsioulpas *et al*., 2007). These differences are probably a consequence of the fact that casein binds calcium and inorganic phosphorus and is separated from whey by addition of rennet and centrifugation, which significantly reduces the amount of these two minerals in colostrum whey as compared to whole colostrum.

In addition, Klimes *et al*. (1986) failed to find a correlation between calcium and phosphorus content and protein concentration in bovine colostrum whey; this is in contrast with the results reported by Gaucheron (2005), who found a significant positive correlation between these parameters in whole milk. These findings lead to the conclusion that the correlation between calcium and inorganic phosphorus and protein fraction of colostrum and milk is due to the interactions between these minerals and casein.

The magnesium content of colostrum whey was higher in group 2 than in the remaining groups and gradually decreased after the onset of lactation (Table 1). Magnesium levels found in group 2 are similar to those reported by Kume and Tanabe (1993). Apparently, the concentration of this mineral is not affected by casein removal.

Table 1. Mean ± standard error (SE) of biochemical parameters of colostral and milk whey of beef cows at first (G1), second (G2), third+fourth (G3), fifth (G4), and sixth (G5) lactations on days 0, 1, 2, 7, 15, and 30 postpartum

Time/ Group	0	1	2	7	15	30
Variable	Gamma-glutamyltransferase (U/L)					
G1	37,496±9,892Aa	16,011±3,957Ab	11,760±3,796Abc	4,088±240Ac	3,716±369Ac	3,322±341Ac
G2	35,903±6,534Aa	14,153±2,410Ab	8,131±950Ab	4,918±294Ab	3,913±369Ab	4,022±328Ab
G3	27,596±6,035Aa	8,776±1,973Ab	5,202±531Ab	4,765±553Ab	3,388±303Ab	3,607±377Ab
G4	27,150±4,152Aa	8,731±705Ab	5,093±421Ab	4,350±311Ab	4,241±456Ab	3,148±277Ab
G5	27,650±3,995Aa	12,403±1,062Ab	6,929±685Ab	4,022±320Ab	3,869±385Ab	3,366±440Ab
	Total protein (g/dL)					
G1	14.5±1.63ABa	5.92±1.71Ab	3.77±1.04Abc	1.32±0.06Ac	1.13±0.05Ac	1.04±0.06Ac
G2	16.9±2.24Aa	3.69±0.63Ab	2.26±0.22Ab	1.52±0.04Ab	1.35±0.01Ab	1.16±0.03Ab
G3	15.1±2.28ABa	3.35±1.10Ab	1.96±0.12Ab	1.41±0.08Ab	1.26±0.06Ab	1.17±0.07Ab
G4	16.4±0.98Aa	3.57±0.62Ab	1.84±0.12Ab	1.41±0.11Ab	1.26±0.06Ab	1.07±0.06Ab
G5	13.0±0.91Ba	4.97±1.16Ab	2.47±0.41Abc	1.50±0.07Ac	1.27±0.07Ac	1.23±0.04Ac
	Calcium (mg/dL)					
G1	55.5±3.00Aa	53.2±2.50Aa	51.7±2.75Aab	44.5±1.45Abc	40.2±1.66Ac	39.4±2.73Ac
G2	55.8±4.49Aa	52.9±2.50Aa	50.6±3.71Aab	50.0±2.40Aab	48.0±1.63Aab	43.7±2.86Ab
G3	50.7±1.87Aa	52.0±3.44Aa	50.4±2.58Aa	48.2±1.78Aab	41.1±1.91Ab	40.5±2.76Ab
G4	51.5±1.94Aa	48.0±1.76Aab	43.5±1.95Aabc	45.3±2.92Aabc	42.8±1.07Abc	39.3±3.87Ac
G5	54.8±2.63Aa	53.0±2.87Aab	53.5±3.39Aa	51.7±4.43Aabc	43.8±1.45Ac	44.7±3.18Abc
	Ionized calcium (mMol/L)					
G1	6.90±0.33ABa	6.16±0.20Aab	5.46±0.15Ab	4.25±0.12Ac	3.65±0.21Ac	3.50±0.11Ac
G2	7.90±0.33Aa	5.70±0.44Ab	5.56±0.27Abc	4.57±0.40Acd	4.32±0.19Ad	4.01±0.22Ad
G3	6.55±0.41Ba	5.85±0.31Aab	5.85±0.55Aab	5.00±0.28Ab	3.89±0.19Ac	3.93±0.25Ac
G4	6.60±0.37Ba	5.49±0.25Ab	4.83±0.19Abc	4.39±0.26Ac	4.09±0.24Ac	3.97±0.19Ac
G5	6.94±0.59ABa	6.00±0.42Aab	5.54±0.19Abc	4.59±0.29Acd	4.41±0.16Ad	3.98±0.18Ad
	Phosphorus (mg/dL)					
G1	39.1±4.39Aa	35.5±2.87Aa	33.7±3.10Aa	37.3±1.34Aa	40.1±2.05Aa	37.4±2.70Aa
G2	47.7±3.06Aa	41.4±2.53Aab	35.7±2.02Ab	33.6±2.21Ab	40.8±3.27Aab	39.1±1.57Aab
G3	37.5±2.81Aa	40.6±2.29Aa	41.6±5.48Aa	38.6±2.45Aa	38.5±2.60Aa	37.9±2.33Aa
G4	39.4±1.64Aab	40.7±3.29Aa	36.5±2.95Aab	34.8±1.94Aab	35.5±1.93Aab	31.4±2.28Ab
G5	42.0±2.62Aa	40.1±3.09Aa	37.5±1.35Aa	39.3±2.23Aa	36.3±1.73Aa	37.0±2.35Aa
	Magnesium (mg/dL)					
G1	19.8±1.83Ba	11.9±1.48Ab	9.57±0.74Abc	7.61±0.15Ac	6.88±0.27Ac	7.00±0.56Ac
G2	24.3±1.95Aa	13.0±1.63Ab	9.80±0.46Abc	8.69±0.53Ac	8.01±0.29Ac	7.76±0.37Ac
G3	17.2±2.47Ba	9.76±0.63Ab	8.29±0.49Ab	7.86±0.56Ab	6.93±0.38Ab	6.62±0.43Ab
G4	18.9±1.33Ba	10.7±0.78Ab	8.17±0.59Abc	7.79±0.58Abc	7.53±0.60Abc	6.91±0.45Ac
G5	19.5±1.54Ba	12.3±1.59Ab	9.69±0.76Abc	8.52±0.63Ac	7.20±0.34Ac	7.75±0.67Ac

Means followed by different capital letters in the same column and lower case letters in the same row differ by Tukey's test ($P<0.05$).

As in the study of Klimes *et al*. (1986), the levels of constituents such as calcium, magnesium, and inorganic phosphorus were much higher at the first milking postpartum. Kume and Tanabe (1993) reported that the colostrum concentrations of calcium, phosphorus, and magnesium were higher immediately after calving and decreased rapidly in the subsequent 24h, which is in contrast with the findings of the present study in which the concentration of these elements also decreased, but in a much slower fashion.

Interestingly, immunoglobulin A (IgA) levels in colostrum whey of group 1 were higher as compared to group 2 (Table 2). The highest concentration of this protein was observed on the day of calving and decreased after the onset of lactation in all groups. Muller and Ellinger (1981) reported that first calf heifers produced a lower level of colostral IgA than third or later lactation cows; these findings differ from the results of the present study.

Table 2. Mean±standard error (SE) of proteinogram of colostral and milk whey in beef cows of first (G1), second (G2), third+fourth (G3), fifth (G4), and sixth (G5) lactations on days 0, 1, 2, 7, 15, and 30 postpartum

Time/ Group	0	1	2	7	15	30
Variable	Gamma-glutamyltransferase (U/L)					
G1	765±175Aa	144±54.6Ab	242±96.6Ab	9.48±2.30Ab	8.03±3.40Ab	1.55±1.02Ab
G2	465±89.1Ba	205±68.1Aab	81.1±24.3Ab	11.0±1.24Ab	5.96±1.64Ab	4.87±3.04Ab
G3	563±188ABa	213±148Ab	41.3±11.4Ab	7.90±3.06Ab	4.35±1.41Ab	3.62±1.48Ab
G4	661±105ABa	182±51.2Ab	40.1±11.5Ab	7.94±4.56Ab	9.16±4.86Ab	4.56±3.04Ab
G5	546±91.1ABa	235±46.6Ab	104±36.5Ab	7.37±2.16Ab	6.72±3.03Ab	3.59±1.18Ab
	Lactoferrin (mg/dL)					
G1	414±72.7Ba	153±51.9Ab	88.5±41.2Ab	17.8±4.00Ab	13.1±4.68Ab	21.9±7.44Ab
G2	731±150Aa	102±28.6Ab	48.5±11.3Ab	40.5±12.3Ab	40.7±14.3Ab	14.9±3.24Ab
G3	559±153ABa	73.1±41.9Ab	29.8±7.05Ab	21.8±7.71Ab	15.1±2.50Ab	14.3±2.24Ab
G4	621±122ABa	67.1±12.3Ab	26.7±4.46Ab	62.9±51.5Ab	13.4±6.73Ab	17.0±4.31Ab
G5	470±83.4Ba	151±60.7Ab	45.4±11.0Ab	27.2±8.55Ab	20.3±6.71Ab	12.9±2.99Ab
	Heavy chain immunoglobulin G (mg/dL)					
G1	4,057±813Aa	763±245Ab	1,033±378Ab	21.7±6.29Ab	11.1±6.47Ab	10.5±5.63Ab
G2	3,597±609Aa	1,094±304Ab	494±111Ab	103±15.4Ab	65.5±9.13Ab	27.7±5.35Ab
G3	3,506±573Aa	759±391Ab	228±49.0Ab	65.3±25.1Ab	31.4±13.0Ab	21.5±6.45Ab
G4	3,953±629Aa	981±289Ab	220±54.4Ab	64.5±31.5Ab	43.4±18.3Ab	19.6±8.87Ab
G5	3,343±381Aa	1,312±341Ab	541±207Abc	72.0±20.1Ac	36.9±9.95Ac	19.9±5.28Ac
	Light chain immunoglobulin G (mg/dL)					
G1	4,276±631Aa	1,151±402Ab	645±253Ab	28.2±6.08Ab	12.0±6.01Ab	17.5±10.1Ab
G2	4,477±916Aa	601±158Ab	242±58.8Ab	38.3±5.37Ab	23.1±4.98Ab	7.16±4.01Ab
G3	4,464±856Aa	539±326Ab	129±25.9Ab	27.9±9.23Ab	10.5±6.09Ab	11.4±4.77Ab
G4	4,782±362Aa	578±168Ab	128±28.7Ab	29.6±14.9Ab	17.0±11.1Ab	8.66±4.82Ab
G5	3,767±337Aa	1,124±418Ab	294±113Ab	37.3±7.98Ab	18.1±3.90Ab	3.53±3.53Ab
	β-Lactoglobulin (mg/dL)					
G1	4,080±843Ba	3,134±1,268Aa	1,326±229Ab	973±55.7Ab	847±46.8Ab	720±81.9Ab
G2	5,788±1,048Aa	1,224±136Bb	1,030±53.1Ab	1,061±49.3Ab	959±32.8Ab	873±35.8Ab
G3	4,967±1,056AB a	1,342±159Bb	1,217±65.6Ab	1,048±43.7Ab	958±37.3Ab	891±48.1Ab
G4	5,144±478ABa	1,334±121Bb	1,092±64.9Ab	986±38.7Ab	943±28.5Ab	824±42.3Ab
G5	3,847±429Ba	1,578±267ABb	1,107±54.2Ab	1,069±61.8Ab	915±56.7Ab	929±55.7Ab
	α-Lactalbumin (mg/dL)					
G1	241±78.2Bab	336±41.7Aa	268±32.9Aab	198±27,3Aab	193±28.1Aab	156±28.6Ab
G2	582±156Aa	232±35.4Ab	259±17.6Ab	200±14.2Ab	181±18.1Ab	162±12.3Ab
G3	354±88.1Ba	297±28.6Aab	251±39.4Aab	187±24.7Aab	188±19.5Aab	174±14.4Ab
G4	418±83.3ABa	273±22.9Aab	261±13.5Aab	196±17.4Ab	182±8.05Ab	151±6.33Ab
G5	346±66.4Ba	280±13.2Aa	276±29.5Aa	227±21.5Aa	204±20.5Aa	212±18.9Aa

Means followed by different capital letters in the same column and lower case letters in the same row differ by Tukey's test ($P<0.05$).

Lactoferrin concentrations in whey from both colostrum and milk were lower in groups 1 and 5 and higher in group 2 (Table 2). The concentrations of this protein also decreased after the onset of lactation in all groups. Tsuji *et al*. (1990) found no significant differences regarding lactoferrin levels between colostrum of primiparous and multiparous beef cows, which differ from the results of the present. In dairy cows, however, these authors observed a lower lactoferrin content in colostrum of first lactation cows as compared to multiparous cows, which is in agreement with the results of the present study. These authors also reported the presence of significantly higher lactoferrin content in the colostrum produced by dairy cows than in that produced by beef cows.

Heavy chain immunoglobulin G (IgG) concentrations did not differ between groups; they were higher on the day of calving and decreased after the onset of lactation (Table 2). Light chain IgG concentrations exhibited the same trend during the experimental period. Sant'ana (2004) reported intermediate immunoglobulin concentrations in first lactation heifers, lower values in second and third lactation cows, and higher values in fourth or later lactation cows.

Colostral beta-lactoglobulin (β-LG) concentrations were lower in group 1 and 5 and higher in group 2 (Table 2). There was also a difference between groups on the first day after calving, when the highest concentration of this protein was observed in group 1. Differently from the present study, Sant'ana (2004) observed a higher concentration of β-LG in colostrum whey of primiparous cows compared with cows in the fourth or later lactations.

Alpha-lactalbumin (α-LA) concentration in colostrum whey was higher in group 2 and lower in groups 1, 3, and 5 (Table 2). A small decrease in the concentration of this protein was observed in the first thirty days of lactation. Sant'ana (2004) observed the highest concentrations of α-lactalbumin in primiparous cows, which is in contrast with the findings of the present study.

The number of cows used in this study was small and it is likely that grouping these animals as primiparous *versus* multiparous would have yielded different results; therefore, further research with larger numbers of cows are warranted.

The concentrations of GGT , immunoglobulin G (both heavy and light chains), calcium, and phosphorus were not influenced by parity in Canchim cows. Concentrations of total protein, lactoferrin, β-lactoglobulin, α-lactalbumin, ionized calcium, and magnesium were higher in multiparous cows, whilst immunoglobulin A concentration was higher in primiparous cows.

Keywords: beef cows, biochemistry, parity, colostrum, milk

RESUMO

Avaliou-se a influência do número de parições nos valores de alguns parâmetros bioquímicos e do perfil eletroforético do soro lácteo de vacas de corte. Trinta e cinco vacas da raça Canchim foram alocadas em cinco grupos: vacas de primeira lactação, segunda lactação, terceira e quarta lactações, quinta lactação e sexta lactação. As amostras de secreção láctea foram coletadas imediatamente após (dia 0) e 1, 2, 7, 15 e 30 dias após o parto. As concentrações de gamaglutamiltranferase (GGT), proteína total, cálcio, fósforo, magnésio e cálcio ionizado foram avaliadas. A separação eletroforética das proteínas foi realizada em matriz de gel de poliacrilamida (SDS-PAGE). A atividade de GGT e as concentrações de imunoglobulina G, cálcio e fósforo não foram influenciadas pelo número de parições. As concentrações de proteína total, cálcio ionizado, magnésio imunoglobulina A, lactoferrina, β-lactoglobulina e α-lactoalbumina, foram influenciadas pelo número de partos das vacas. À exceção dos teores de fósforo e α-lactoalbumina em poucos grupos, a concentração das demais características decresceu no decorrer do período de lactação.

Palavras-chave: vaca de corte, bioquímica, ordem de parição, colostro, leite

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