Casein to Whey Protein Ratio in Rat and Human Milks: Effects of Maternal Protein Intake

PATRICIA A. RONAYNE DE FERRER and MARIA ELENA SAMBUCETTI
Department of Nutrition and Food Science
School of Pharmacy and Biochemistry
University of Buenos Aires
Buenos Aires, Argentina

ABSTRACT
Maternal diet may affect milk composition quantitatively and qualitatively. The aim of this study was to assess the effects of different dietary protein quality and quantity on total milk protein concentration and on possible changes in casein:whey protein ratio as a result of differences in maternal nutritional status. Milk samples were obtained from Wistar rats fed either wheat gluten or supplemented casein at different percentages. A similar analytical scheme was applied to milk samples from lactating women belonging to different socioeconomic classes. Total milk protein in rat milk was related closely to protein quality and, to a lesser extent, to protein quantity, showing significant differences among treatment groups. Relative proportions between milk proteins were significantly affected by diet: casein:whey protein ratio was related positively to protein quality and quantity. In human milk, even though total protein content did not differ, the relative proportions between milk proteins were altered, but in a way opposite to that observed in rat milk: the whey protein:casein ratio was related positively to maternal protein intake. These modifications might influence the nutritive value or antiinfectious properties of the milk ingested by the neonate.

(Key words: casein, whey proteins, protein intake)

INTRODUCTION
Recently, interest has been renewed in breast feeding, and human lactation is now receiving increased scientific attention. Of special concern is malnutrition, which can affect lactational performance and milk composition; thus, research efforts on this subject are increasing worldwide, particularly in developing countries. Some studies (7, 10) relating maternal nutritional status and dietary intake to milk composition in humans have led to contradictory conclusions, especially with regard to protein intake. To clarify these relationships, several investigators have approached this problem using the rat as a model. Their studies have examined the effect of dietary protein quality, protein quantity, and food intake on protein synthesis and mammary tissue composition (27) or the effect of maternal diet on protein synthesis, milk production, and milk composition (11). In a preliminary study (26) on rat milk composition, we found a correlation between maternal protein intake and total milk protein.

However, a relevant point that generally has been overlooked is the effect of diet on qualitative changes of milk proteins. Relative proportions of milk proteins may differ according to maternal nutritional status in rats (29) and in humans (6), but effects on individual proteins were not studied extensively.

The present study examines the effects of different dietary protein quality and quantity on total milk protein concentration and on possible changes in casein:whey protein (CN:WP) ratio. The same scheme was applied to milk samples obtained from lactating women belonging to different socioeconomic classes.

Abbreviation key: CN = casein, CN-M = CN supplemented with Met, TN = total N, WG = wheat gluten, WP = whey protein.
MATERIALS AND METHODS

Study in Rats

Rats and Diets. Female Wistar rats weighing 180 g were bred with males of the same strain. When pregnancy was confirmed, dams were housed in individual stainless-steel wire-bottomed cages. Throughout the study, the rats were maintained in a room with automatically controlled lighting (light cycle: 0800 to 2000 h) with standardized temperature (21°C).

Feed and water were available for ad libitum intake. Rats were fed stock diet (Cargill, Buenos Aires, Argentina) until parturition. From d 1 of lactation (day after parturition), dams were divided into five groups of 10 lactating dams each and fed the experimental diets (Table 1). Litter size was adjusted to 6 to 8 pups on d 1 of lactation. The treatment groups varied in dietary protein quality [wheat gluten (WG) or CN supplemented with Met (CN-M)] and protein quantity, as follows: WG at 12.6 and 17.8% and CN-M at 8.04, 13.7, and 17.7%. Because diets were isocaloric, protein quantities by weight also corresponded to percentage of protein calories. The WG was not tested at 8% because dams would not be able to raise litters successfully at that percentage (13).

Intake Records. Feed intake was recorded daily, and energy and protein intakes were calculated. For each treatment group, we calculated the intake of "complete" protein (amount of utilizable protein according to essential AA composition). The AA content of CN and WG were taken from published data (8). The standard employed to calculate chemical scores was the list of AA requirements for lactation (23). The chemical scores were 100 for CN-M and 40 for WG, which are typical for biological evaluations carried out in our department.

Sample Collection. Milk collection was carried out at d 15 of lactation. Dams were anesthetized with sodium Pentothal® (Abbott Laboratories, Buenos Aires, Argentina) by intraperitoneal injection of 10 to 12 mg per dam. Oxytocin (.1 IU per dam; Syntocinon Sandoz, 25 ancor · Rafaela, Santa Fe, Argentina. Protein content, 80.4%.

3Nutritional Biochemical Corporation, Cleveland, OH.
4Refinerias de maiz, Baradero, Argentina.
5Contained (grams per kilogram) thiamin-HCl, 2.0; riboflavin, 2.0; nicotinic acid, 10.0; pyridoxine-HCl, 1.0; folic acid, .08; D-biotin, .04; D-calcium pantothenate, 8.0; cyanocobalamin, .06; inositol, 40.0; ascorbic acid, 20.0; and sucrose to make 1000.0 g (16).
6Contained cholecalciferol, 2000 IU; retinol, 4000 IU; α-tocopherol, 100 mg; and oil to make 1000 g (this mix was incorporated into corn oil) (16).

TABLE 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Glutens</th>
<th>Casein + methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.6%</td>
<td>17.8%</td>
</tr>
<tr>
<td>Wheat gluten¹</td>
<td>17.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Casein²</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>Methionine³</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>Dextrin⁴</td>
<td>68.65</td>
<td>61.65</td>
</tr>
<tr>
<td>Water-soluble vitamin mix⁵</td>
<td>.25</td>
<td>.25</td>
</tr>
<tr>
<td>Fat-soluble vitamin mix⁶</td>
<td>.5</td>
<td>.5</td>
</tr>
<tr>
<td>Salt mix⁷</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>.6</td>
<td>.6</td>
</tr>
<tr>
<td>Corn oil</td>
<td>10.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

¹Melrico, Buenos Aires, Argentina. Protein content, 74.2%.
²Sancer, Rafaela, Santa Fe, Argentina. Protein content, 80.4%.
³Nutritional Biochemical Corporation, Cleveland, OH.
⁴Refinerias de maiz, Baradero, Argentina.
⁵Contained (grams per kilogram) thiamin-HCl, 2.0; riboflavin, 2.0; nicotinic acid, 10.0; pyridoxine-HCl, 1.0; folic acid, .08; D-biotin, .04; D-calcium pantothenate, 8.0; cyanocobalamin, .06; inositol, 40.0; ascorbic acid, 20.0; and sucrose to make 1000.0 g (16).
⁶Contained cholecalciferol, 2000 IU; retinol, 4000 IU; α-tocopherol, 100 mg; and oil to make 1000 g (this mix was incorporated into corn oil) (16).
⁷Contained (grams per kilogram) CaHPO₄, 570; NaCl, 74; potassium citrate (monohydrate), 220; K₂SO₄, 52; KH₂PO₄, 45.4; MgO, 24; MnSO₄·H₂O, 4.75; ammonium ferric citrate, 5.9; ZnSO₄·7H₂O, 3.8; CuSO₄·5H₂O, .67; KIO₃, .01; SeO₂, .004; according to AIN76 mineral mix, except that KH₂PO₄ was included and CaHPO₄ was added in larger amounts to meet American Institute of Nutrition recommended levels for lactating rats for Ca, P, and K (1, 2).

Journal of Dairy Science Vol. 76, No. 6, 1993
Buenos Aires, Argentina) was injected intraperitoneally to stimulate milk flow. Milk samples were obtained by hand stripping of teats. Droplets were collected into capillary tubes, which were emptied into Eppendorf polypropylene tubes and kept at -20°C until analysis. Collection time for each dam was about 30 min. Dams always were milked between 1200 and 1400 h to minimize possible diurnal variations in milk composition (15). Milk collection was carried out within 1 h after dams and pups were separated to avoid compositional changes because of milk stasis (12). Samples were analyzed for protein content, electrophoretic profile, and Na and K concentrations.

Analytical Methods. Total N (TN) was assessed by the Kjeldahl method (4). Protein content was calculated as (TN × 6.25). Sodium and K were determined by flame photometry with a 598.3-nm filter for Na and a 766.5-nm filter for K (Flame Photometer Crudo Caamaño model 501; Buenos Aires, Argentina). Samples were diluted 1:80 (vol/vol) for Na and 1:90 (vol/vol) for K.

Electrophoresis: Sample Treatment. Prior to electrophoretic separation, samples were defatted by centrifugation at 300 × g for 30 min. The infranatant solution was extracted according to the method of Lee et al. (18) with .0625 M Tris·HCl buffer, pH 6.8, containing 3% SDS and 1% mercaptoethanol. The mixture was placed in a boiling water bath for 5 min.

Electrophoretic System. Electrophoresis was carried out by the discontinuous system described by Laemmli (17). Acrylamide gels of 10% (37:1, wt/wt, acrylamide to bisacrylamide) were prepared between glass plates measuring 175 × 80 mm. Polymerization agents were ammonium persulfate and N,N,N,N-tetramethylethylenediamine. The buffers employed were 1) stacking gel, .5 M Tris·HCl, .4% SDS, pH 6.8; 2) separation gel, 1.5 M Tris·HCl, .4% SDS, pH 9.2; and 3) buffer solution of the electrode reservoir, .025 M Tris, .192 M glycine, .1% SDS, pH 8.3.

Samples were mixed with 30% glycerol and bromophenol blue prior to application on the gels. When migration was completed, proteins were fixed with 15% TCA acid for 30 min; this step also removed SDS, which interferes with the staining. The gels were stained with Coomassie brilliant blue R 250 (Mallinckrodt Chemical Works, USA, fractionated in Argentina) and scanned at 550 nm.

Calculation of Protein Amounts. Approximate values for CN, α-lactalbumin, transferrin, and serum albumin were calculated from densitometric tracings of pooled samples subjected to electrophoretic separation on SDS-polyacrylamide gels. Even though this procedure did not yield absolute values, and proteins may have differed in dye-binding capacities, we found the procedure to be sufficiently valid for a comparison among different treatment groups. Each peak was identified as described previously (25). Each peak area was calculated as peak height times width at half-height (14). Because the total area corresponded to total protein content, we assessed each protein amount from its relative area. To test linearity of the densitometer responses, we plotted milk protein areas versus sample loading (volumes employed were 10, 20, 25, and 30 μl) and obtained the following correlation coefficients: transferrin, r = .99; serum albumin, r = .91; α-lactalbumin, r = .84; and CN, r = .95.

Study in Humans

Subjects and Samples. Two groups of lactating humans were studied. Group 1 consisted of 19 women who belonged to the middle class (79 and 47% of them had completed high school and university careers, respectively; their husbands were employed full-time). Group 2 consisted of 19 women who belonged to a low income community (43 and 28% of them had completed elementary and high school, respectively; their husbands worked occasionally).

Milk samples were obtained by total manual expression of one breast, collected in sterile plastic containers, and kept at -20°C until analysis. All collections were made between 1000 and 1200 h and between d 14 and 30 postpartum. Samples were analyzed for TN and NPN. A pooled sample was obtained from each group and subjected to electrophoresis. Dietary intake was estimated from 24-h recall using a 1-d record form.

Analytical Methods. Nitrogen content was assessed by the Kjeldahl method (4). The TN and NPN were determined; NPN was obtained by protein precipitation with 24% TCA acid (21). Protein N was calculated as (TN - NPN).
Protein content was calculated as (protein N x 6.25).

Electrophoresis. Electrophoresis was carried out as previously described.

Calculation of Areas. The areas corresponding to the peaks obtained in densitometric tracings were calculated by being cut and weighed on an analytical scale (14). Measured areas were those corresponding either to β-CN or to the sum of the remaining protein fractions.

Dietary Records Analysis. Protein intakes were calculated from the 1-d dietary records using a computer program that had been loaded with food composition data from different sources (19, 28).

Statistical Analyses

Data were analyzed by one- or two-way ANOVA followed by Duncan's multiple comparison test. Correlations between variables were calculated by simple linear regression analysis. Significance was declared at $P < .01$ unless otherwise noted.

RESULTS

Study in Rats

Food intake of dams, total protein intake, and "complete" protein intake are listed in Table 2. Table 3 shows total milk protein, CN, and WP concentrations in rat milk. Total milk protein concentrations were related to different dietary treatments. Dams fed WG produced milk that had the lowest protein content, regardless of WG intake, and that was similar to protein content for dams fed 8.04% CN-M. The protein amounts were highest when dams were fed 13.7 and 17.7% CN-M and were significantly greater ($P < .01$) than the others.

Regarding protein fractions, milk CN showed modifications closely related to total protein. The WP were apparently independent of diet.

Electrophoretic separations of milk protein corresponding to the five dietary treatments are shown in Figure 1. The analysis of the densitometric tracings revealed that relative proportions of proteins differed among groups (Table 4). The most remarkable findings were that CN areas were correlated positively with protein quality and quantity, whereas the serum albumin area was correlated inversely to them. Total WP followed the same pattern as serum albumin because it is the most abundant WP. The CN:WP ratio increased significantly when protein content was higher, maintaining constant protein quality (12.6 vs. 17.8% WG and 8.04 or 13.7 vs. 17.7% CN-M). The remarkable difference between 12.6% WG and 17.7% CN-M shows the combined effect of protein quality and quantity.

Study in Humans

The analysis of the intake records showed that the protein intakes of the two groups of lactating humans were significantly different (Table 5). However, protein requirements were

| TABLE 2. Dams' food, total protein, and complete protein intakes. |
|---|---|---|
| Diet1 (%) | Food intake (as fed) | Protein intake |
| | $\bar{X}$ | SEM | $\bar{X}$ | SEM |
| WG | 12.6 | 20.7* | .8 | 2.61* | .11 | 1.04* | .04 |
| | 17.8 | 23.6* | 1.2 | 4.20* | .22 | 1.68* | .09 |
| CN-M | 8.04 | 24.5* | 1.1 | 1.97* | .09 | 1.97* | .09 |
| | 13.7 | 27.8* | 1.5 | 3.80* | .20 | 3.80* | .20 |
| | 17.7 | 27.9* | 1.6 | 4.78* | .29 | 4.78* | .29 |

*Means within columns not sharing a common superscript differ ($P < .01$).

1WG = Wheat gluten; CN-M = casein supplemented with Met; percentage in basal diet.

2Complete protein = utilizable protein.
TABLE 3. Concentrations of total protein (TP), casein (CN), serum albumin (SA), transferrin (TF), and α-lactalbumin (α-LA) in rat milk.

<table>
<thead>
<tr>
<th>Diet</th>
<th>TP (%)</th>
<th>CN (%)</th>
<th>SA (%)</th>
<th>TF (mg/ml)</th>
<th>α-LA (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SEM</td>
<td>X</td>
<td>SEM</td>
<td>X</td>
</tr>
<tr>
<td>WG</td>
<td>12.6</td>
<td>68.9b</td>
<td>44.6b</td>
<td>.6</td>
<td>12.0a</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>68.0e</td>
<td>49.4e</td>
<td>1.6</td>
<td>8.1c</td>
</tr>
<tr>
<td>CN-M</td>
<td>8.04</td>
<td>73.3b</td>
<td>51.2c</td>
<td>.4</td>
<td>10.5ab</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>92.8b</td>
<td>64.3b</td>
<td>.3</td>
<td>11.7b</td>
</tr>
<tr>
<td></td>
<td>17.7</td>
<td>90.6a</td>
<td>68.6a</td>
<td>.2</td>
<td>9.0a</td>
</tr>
</tbody>
</table>

Means within columns not sharing a common superscript differ (P < .01).

(a,b,c,d)Means within columns not sharing a common superscript differ (P < .01).

1WG = Wheat gluten; CN-M = casein supplemented with Met; percentage in basal diet.

Figure 1. Milk protein electrophoretic separations corresponding to the following experimental diets: 1) casein (CN), 8.04%; 2) CN, 13.7%; 3) CN, 17.7%; 4) wheat gluten (WG), 12.6%, and 5) WG, 17.8%. The main bands are transferrin (TF), serum albumin (SA), CN, and α-lactalbumin (α-LA).
met in both groups, even in the low income group (9). Because essential AA needs are higher during lactation, we calculated not only total protein intake but also the daily intake of essential AA. No deficiencies occurred in either group (data not shown).

When total milk protein was determined, no significant difference appeared between groups (Table 5), even though maternal protein intakes were different.

Relative proportions of milk proteins differed between groups, as seen in Figure 2. Measured areas were those of β-CN and the sum of the remaining areas. Because β-CN is the major subunit of this protein fraction, the following approximation was made: the β-CN area was considered to be representative of total CN, and the remaining area was considered to be WP (Table 6). Thus, a portion of κ-CN might have been included in the area defined as WP. However, the error was not great because κ-CN was minimal compared with total WP.

These results showed that the WP area was directly proportional to maternal protein intake, and the CN-M area varied inversely. Consequently, the WP:CN ratio was related positively to maternal diet, because it increased significantly ($P < .01$) with increased maternal protein intake. We did not employ the CN:WP ratio, as in rat milk, because WP are the major fraction of total proteins in human milk. Serum albumin proportions, as estimated from densitometric tracings, were not significantly different (Table 6).

**DISCUSSION**

**Study in Rats**

The effects of dietary protein on milk composition were noteworthy. Total protein was related closely to protein quality and, to a lesser extent, to protein quantity. The decrease in dietary protein to 8.04% CN-M caused a reduction of about 20% in milk protein content, which is similar to data reported by Sturman et al. (29), who found a 30% reduction when dietary CN diminished from 25 to 6%.

Crnic and Chase (5) also found that milk N decreased about 20% when dietary CN was reduced from 27 to 8%. These changes were attributable mainly to CN variations, which constituted about 65 to 75% of total protein.

![Figure 2. Milk protein electrophoretic separations and densitometric tracings from group 1 (middle class women) (A) and group 2 (low income women) (B). The peak corresponds to β-casein (β-CN).](image-url)
TABLE 4. Percentages of areas from densitometric tracings of protein electrophoretic separations in rat milk.1

<table>
<thead>
<tr>
<th>Diet</th>
<th>CN (%)</th>
<th>SEM</th>
<th>SA (%)</th>
<th>SEM</th>
<th>WP (%)</th>
<th>SEM</th>
<th>CN:WP (%)</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WG</td>
<td>12.6</td>
<td>64.8a</td>
<td>.9</td>
<td>17.35a</td>
<td>.45</td>
<td>27.6a</td>
<td>1.05</td>
<td>2.35c</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>72.6a</td>
<td>2.2</td>
<td>11.93bc</td>
<td>1.05</td>
<td>21.8bc</td>
<td>1.55</td>
<td>3.36bc</td>
</tr>
<tr>
<td>CN·M</td>
<td>8.04</td>
<td>70.0bc</td>
<td>.6</td>
<td>14.30b</td>
<td>.80</td>
<td>23.6b</td>
<td>.30</td>
<td>2.96bc</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
<td>69.5c</td>
<td>.3</td>
<td>12.55bc</td>
<td>.05</td>
<td>23.2bc</td>
<td>.25</td>
<td>3.00bc</td>
</tr>
<tr>
<td></td>
<td>17.7</td>
<td>75.6a</td>
<td>.2</td>
<td>9.95c</td>
<td>.05</td>
<td>19.1c</td>
<td>.60</td>
<td>3.96a</td>
</tr>
</tbody>
</table>

Means within columns not sharing a common superscript differ (P < .01).

1CN = Casein; WP = whey protein; SA = serum albumin; WG = wheat gluten; CN·M = CN supplemented with Met.

Regarding the relative proportions of milk proteins, our results demonstrated that CN and serum albumin proportions were affected significantly by diet; thus, milk produced by dams subjected to different dietary treatments differed in the CN:WP ratio. Others (24) have shown that milk composition is affected greatly in certain situations, such as the beginning and end of lactation (weaning), during prolonged food deprivation, and during mastitis. These modifications have been attributed to the opening of a paracellular pathway, which permits a direct exchange between extracellular fluid and milk. This exchange induces an increase in WP (e.g., serum albumin) and Na and a decrease in lactose and K concentrations (3).

The results obtained in our study suggest the following hypothesis: because of the nutritional stress produced by insufficient dietary protein (both quality and quantity), epithelial cells lose their functional integrity, thus provoking a diminished synthesis of specific components (lactose and proteins of mammary origin, such as CN) and an opening of the paracellular pathway because of leaky junctions between cells. The modifications observed in CN:WP ratio agree with a functioning paracellular pathway when diets were of low quality, as shown by the measurement of Na and K ions (Table 7). Milk from dams fed 12.6% WG had the highest concentrations of Na and serum albumin and the lowest concentrations of K and CN. In addition, serum albumin concentrations were correlated positively to Na concentration (r = .99) and negatively with K concentrations (r = -.92), according to what might be expected when the paracellular pathway is open.

Study in Humans

In spite of the significant difference in maternal protein intakes, total milk protein was similar in both groups, probably because protein requirements were met even in the low income group. Forsum and Lönnérdal (10) found differences in TN, protein N, and NPN contents in milk from humans that ate diets providing either 8 or 20% of the calories in protein. This increase in N content mainly was due to urea N, but no significant differences were found in daily secretions of α-lactalbumin, lactoferrin, and serum albumin.

Other studies (6, 22) have indicated that curd:WP ratio in human milk was related to maternal nutritional status. Malnourished lactating women showed higher ratios, which led to the hypothesis that WP and NPN concentrations were lower. Those papers (6, 22) were
published about 30 yr ago, and, in spite of the potential consequences, this subject has not been well researched, probably because of the difficulties inherent in obtaining reliable quantitative measurement of CN in human milk (20).

Because these modifications in milk protein proportions might influence the nutritive value, anti-infectious properties, or both of milk ingested by the newborn, we resumed this investigation. Even though the methodology used in this study based on electrophoretic profiles analysis does not lead to total quantitative measurement of CN, it permits an estimation of the relative changes between CN and the remaining proteins. Thus, we could establish that the variations observed with lower protein intake were due to diminished WP and increased CN. These changes indicate that the synthesis of individual milk proteins in the mammary gland is not regulated in the same manner. Because the proportion of serum albumin was the same in both groups, protein transfer from the blood stream was not likely to occur, either by transport across the epithelial cells or by a paracellular pathway. This aspect seems to be opposite to the results obtained in rats, for which the existence of a paracellular pathway was demonstrated.

CONCLUSIONS

Total Protein

Even though our primary conclusion is that, in rats, differences in total milk protein were significant among the experimental groups, this was not the case in humans; this point may merit further study.

Variations in rat milk were mainly due to dietary protein quality and depended on quantity only when protein intake was low (8%). However, when the increases of either dietary protein (WG or CN) from 13 to 17% were compared, milk protein content was not significantly different. The phenomenon was the same in lactating humans because dietary percentages of protein calories were about 17 and 13%, and dietary protein quality was comparable with that of CN-M.

Relative Proportions of Protein Fractions

In rat milk, the CN:WP ratio increased when dietary protein quality and quantity improved, but, in human milk, this ratio diminished when protein intake was higher. The fractions that respond positively to an increase in dietary protein are those that constitute the major fractions in each case, i.e., CN in rat milk and WP in human milk. These changes probably were due to different causes because, in rats, a paracellular pathway was demonstrated, which did not seem to be the case in humans.

These findings stress the importance of further studies on the effects of maternal protein intake on milk proteins because these modifi-
MILK PROTEINS AND MATERNAL PROTEIN INTAKE

1653

ACKNOWLEDGEMENTS

We thank Maria E. Barrio Rendo for her valuable help in milking the rats, Nora López and nutritionist Laura López for the patient collection of human milk samples, and Lía Calafat for her helpful technical assistance.

REFERENCES


Journal of Dairy Science Vol. 76, No. 6, 1993