

## Changes in the chemical status of calcium in casein micelles with the pH of milk

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### 1. Introduction

Colloidal calcium phosphate plays a significant role in the maintenance of the integrity of casein micelles (1). It is well known that most of the colloidal calcium phosphate is converted to the soluble form on lowering the pH of milk (2). Further, the level of calcium in casein micelles in milk from cow, buffalo and goat differs significantly (3, 4). The release of calcium, inorganic phosphate, sialic acid and non-protein nitrogen from buffalo casein micelles on acid treatment was reported earlier from this laboratory (5). However, neither the extent of detachment of calcium from the casein micelles nor from micelles of different size in milk of any of the above species as a function of the pH has been reported so far. For this reason and from a comparative angle, the extent of release of calcium from the casein micelles of milk from these three species is reported here. Such study is expected to give a better understanding of the chemical group involved in the attachment of calcium to the casein micelles.

### 2. Materials and methods

#### 2.1 Milk samples

Pooled milk samples were collected from cow, buffalo and goat maintained at the Institute herd. Skimming was done immediately with the aid of an Alva-Laval cream separator and subsequently the studies were carried out at room temperature.

#### 2.2 Preparation of casein micelles

Either whole casein micelles or the big, medium and small size micelles were obtained by differential ultracentrifugation of skim milk as described by SABARWAL and GANGULI (6). The micellar sediments were then resuspended in an equivalent volume of ultracentrifugal whey corresponding to its original milk volume.

#### 2.3 pH adjustment

Either casein micelles or different size micelles suspended in ultracentrifugal whey were adjusted to various pH values between 6.8 and 4.5 with 1N HCl.

#### 2.4 Turbidity measurement

pH-adjusted big and small size micellar suspensions were centrifuged at 11,000 and 46,000 g for 30 min, respectively. Supernatants thus obtained were carefully removed and turbidity measured in a Klett-Summerson photoelectric colorimeter using red filter. Blank was adjusted with distilled water.

#### 2.5 Micellar casein estimation

An indirect approach for estimating micellar casein on the basis of nitrogen distribution was used. The total nitrogen in milk and in ultracentrifugal whey (105000 g, 30 min) was determined by micro-Kjeldahl method as described by OSER (7). Micellar casein was calculated by subtracting whey value from the corresponding milk value.

#### 2.6 Calcium estimation

Calcium was estimated by the method of DAVIES and WHITE (8) in ultracentrifugal whey for calcium re-

lease studies. Micellar calcium was calculated by subtracting whey calcium value from the corresponding milk value.

#### 2.7 Gel filtration

Gel filtration was carried out according to IMADE *et al.* (9). Sephadex G-25 was packed in 60 x 1 cm. column and equilibrated with 0.01 M Tris HCl buffer pH 7.0, containing 0.07 M KCl and 5 mM CaCl<sub>2</sub>. 10 mg acid casein (prepared by precipitation at pH 4.6) was dissolved in 0.5 ml of above buffer system with stirring for 2-3 h. pH was adjusted to 7.0 with Tris and the sample was applied to the column. The same buffer was used for elution. Seventeen fractions, each of 2.5 ml volume, were collected using a flow rate of 11 ml/hr. Protein was estimated by the method of LOWRY *et al.* (10) using acid casein as standard. Bound calcium was calculated according to HUMMEL and DREYER (11).

### 3. Results and discussion

#### 3.1 The extent of release of calcium from casein micelles with pH

The estimated cow micellar calcium (88.9 mg) and whey calcium (35.5 mg) per 100 ml milk indicated that 71.4 % of total calcium is accountable by the micelles. The studies on the pH-dependent release of calcium from casein micelles of milk revealed almost complete detachment of calcium from the micelles at pH 5.0 (Figure 1). However, the extent of release was more pronounced in the pH range of milk from 6.0 to 5.0. From the calcium release profile with pH it certainly seems that dissociation/association of some of side chain amino acid groups is the key factor in determining the micellar calcium status. The phosphate group probably linked to hydroxyl group of serine and threonine having pK<sub>2</sub> around 6.7 (12) exists undissociated as pH drops, resulting in the release of calcium. The imidazole group (pK around 6.0) of histidine indirectly enhances calcium release as a result of protonation at low pH by which protein molecule acquires a lower negative charge. These results are in agreement with binding studies of DICKSON and PARKINS (13) suggesting the involvement of phosphate and imidazole groups in calcium-casein interaction in this region of pH.

#### 3.2 Calcium release from micelles of different size

Dependence of micellar calcium release on pH for different size micelles is shown in Figure 2. Micellar calcium release was higher from big micelles than either medium or small size micelles. The difference in the extent of release of calcium can be attributed to the differences in the proportions of  $\alpha_s$ ,  $\beta$  and  $\kappa$ -caseins in these different size micelles,  $\alpha_s$ -casein being more in big micelles than in small micelles (14, 15). Further,  $\alpha_s$ -casein constitutes relatively more phosphate groups (pK<sub>2</sub> ~6.7) than any other fractions of casein and thus big micelles become more sensitive to pH changes in this region than either medium or small size micelles.

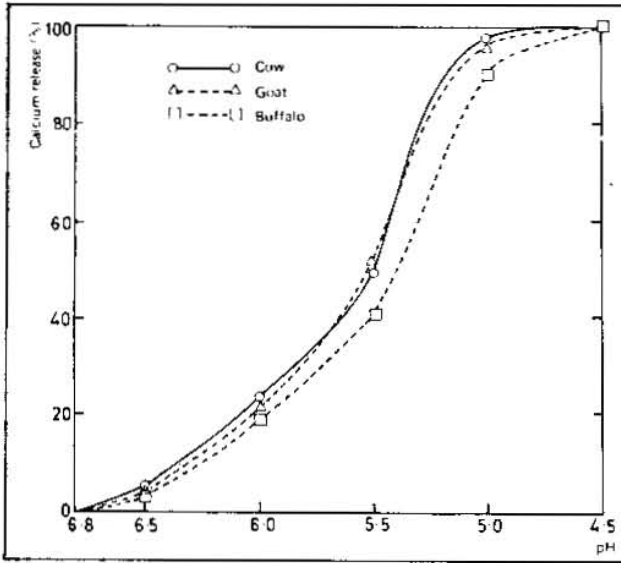


Figure 1: Release of micellar calcium in milk from different species with pH.

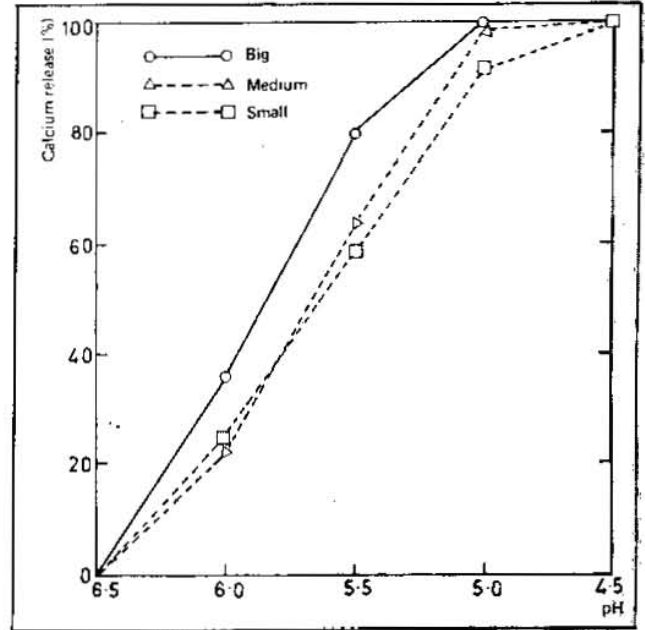


Figure 2: Size dependence on the release of micellar calcium with pH.

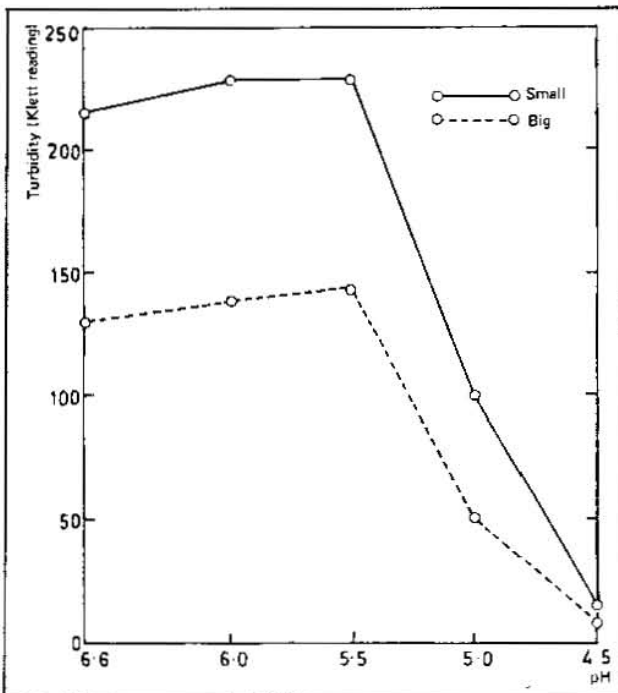


Figure 3: Sedimentation behaviour of casein micelles with change in pH.

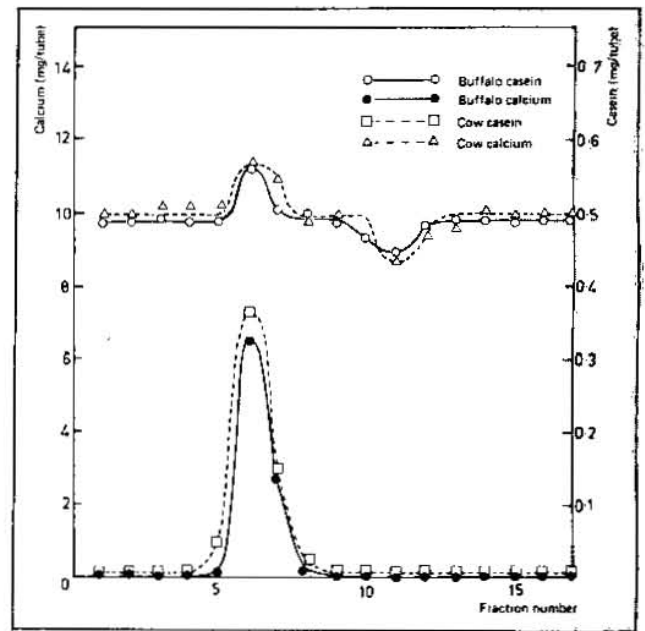


Figure 4: Comparative elution profiles of calcium ion from caseins of cow and buffalo milk.

3.3 Sedimentation behaviour of pH-treated micelles

Turbidity measurement studies with big and small size micelles adjusted to various pH values were carried out to have an idea of sedimentation behaviour of micelles after pH-treatment. Figure 3 illustrates that pH drop to 5.5 did not change turbidity significantly indicating neither dissociation of big micelles nor association of small micelles had occurred but a sharp fall in turbidity was observed at pH 5.0 which was negligible at pH 4.5. This suggests that at pH 5.0 precipitation of casein starts which completes at pH 4.5.

3.4 Calcium-protein relationship

An indirect attempt was made to estimate micellar protein by calculating the calcium/protein ratio for pooled milk samples. The calcium (mg)/micellar casein (g) ratio varied from 34.0 to 38.0 and thus cannot be used for the proposed purpose.

3.5 Micellar calcium release from milk of other species

Results in Table 1 show that milk from buffalo and goat differed from that of cow in the distribution of calcium

between micellar and soluble phases. The calcium ratio micellar to soluble form was higher in buffalo milk than either in cow or goat milk.

The extent of calcium release from cow and goat milk was similar while buffalo milk exhibited lower release (Figure 1) at all pH ranging between 6.8 to 5.0. Since, the proportion of  $\alpha_s$ -casein is lower in buffalo casein than in cow (16), this could probably account for the relatively slower release of calcium from buffalo micelles.

### 3.6 Evaluation of bound calcium

As the release of micellar calcium differed in the case of cow and buffalo milk, the calcium uptake by the casein from these milks was studied. The elution profile for cow and buffalo casein and calcium is shown in Figure 4. Bound calcium for cow and buffalo acid casein was found to be 21.9 g atoms and 20.9 g atoms for  $10^5$  g casein, respectively. The value of bound calcium for bovine casein is higher than reported by *IMADE et al.* (9) and lower than *ZITTLE et al.* (17). The calcium in buffalo casein micelles is relatively more than in cow casein micelles (Table 1). Further calcium uptake by both cow and buffalo acid casein is equal. Since acid casein lacks in some of the interacting sites (5), possibility of more loss of these sites in buffalo acid casein than cow cannot be ruled out.

Table 1: Distribution of calcium in milk whey and casein micelles in cow, buffalo and goat

Species	Average calcium level <sup>1</sup> (mg/100 ml milk)			Calcium ratio (A-B) (B)
	Milk (A)	Ultracentrifugal whey (B)	Casein micelles (A-B)	
Cow	124.4 ± 9.38 <sup>2</sup>	35.5 ± 3.84	88.9 ± 6.30	2.50
Buffalo	209.6 ± 6.79	36.9 ± 6.53	172.7 ± 10.28	4.68
Goat	142.6 ± 12.31	38.5 ± 4.56	104.1 ± 12.23	2.70

<sup>1</sup> The number of samples analysed for cow, buffalo and goat was 7, 7 and 6, respectively.  
<sup>2</sup> Standard deviation

## 4. References

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## 5. Summary

RAJPUT, Y.S., BHAVADASAN, M.K., GANGULI, W.C.: Changes in the chemical status of calcium in casein micelles with the pH of milk. *Milchwissenschaft* 38 (4) 211-213 (1983).

### 34 Casein micelles of milk (calcium release)

The release of calcium from cow casein micelles revealed almost complete detachment of calcium at pH 5.0 which was more pronounced when pH was lowered from 6.0 to 5.0. Decalcification of big micelles was relatively more than either medium or small size micelles as pH was lowered. Turbidity studies indicated neither dissociation of big micelles nor association of small micelles with lowering of pH to 5.5. Micellar calcium and casein ratio varied from sample to sample.

Casein micelles from buffalo milk contained more calcium than either cow or goat milk. Comparative studies on casein micelles from other species revealed that the extent of calcium release from cow and goat milk was similar, while buffalo milk exhibited lower release. Cow and buffalo acid casein had the almost similar binding capacity for calcium at pH 7.0.

RAJPUT, Y.S., BHAVADASAN, M.K., GANGULI, W.C.: Veränderungen im chemischen Zustand von Calcium in Caseinmicellen mit dem pH-Wert von Milch. *Milchwissenschaft* 38 (4) 211-213 (1983).

### 34 Caseinmicellen von Milch (Calciumfreisetzung)

Die Freisetzung von Calcium aus Rinder-caseinmicellen zeigte eine fast vollständige Abgabe des Calcium bei pH 5,0; dies wurde noch deutlicher, wenn der pH von 6,0 auf 5,0 herabgesenkt wurde. Die Calciumabgabe großer Micellen war relativ stärker als die kleiner Micellen. Trübungsuntersuchungen zeigten weder ein Dissoziation großer Micellen noch eine Zusammenlagerung kleiner Micellen, wenn der pH-Wert auf 5,5 gesenkt wurde. Das Verhältnis von micellarem Calcium und Casein variierte von Probe zu Probe.

Caseinmicellen von Büffelmilch enthielten mehr Calcium als Kuh- oder Ziegenmilch. Vergleichende Untersuchungen von Caseinmicellen anderer Species zeigten, daß der Grad der Calciumabgabe von Kuh- und Ziegenmilch ähnlich ist, während Büffelmilch weniger Calcium freisetzt. Säurecasein von Kuh- und Büffelsäure besaßen für Calcium bei pH 7,0 fast gleiches Bindungsvermögen.

RAJPUT, Y.S., BHAVADASAN, M.K., GANGULI, W.C.: Modifications dans l'état chimique de calcium dans des micelles de caséine par la valeur pH du lait. *Milchwissenschaft* 38 (4) 211-213 (1983).

### 34 Micelles de caséine (libération de calcium)

RAJPUT, Y.S., BHAVADASAN, M.K., GANGULI, W.C.: Cambios en el estado químico de calcio en micelas de caseína por el valor pH de leche. *Milchwissenschaft* 38 (4) 211-213 (1983).

### 34 Micelas de caseína (liberación de calcio)