Characterization of Products from in Vivo and in Vitro Gastric Digestion of Milk Replacers Containing Whey Proteins†

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Gastric digestion of three milk replacers containing either total milk proteins or a mixture (50:50) of total milk proteins and native or heated whey protein concentrate was studied in vivo with three preruminant calves and in vitro using rennet in an artificial stomach. Stomach effluents were collected during 6 h in vivo and 3 h in vitro and were analyzed for amino acid composition. Proteins and large peptides were characterized by SDS-PAGE. With the skim milk powder diet, casein coagulated almost immediately and was evacuated later as degraded products. Coagulation of milk replacers containing whey proteins was delayed, and a large amount of proteins and large peptides was evacuated rapidly. The digestions of both milk replacers were similar. In vivo and in vitro methods provided similar results, suggesting that the in vitro method could be used to simulate in vivo gastric digestion.

INTRODUCTION

In the preruminant calf, the first step of milk digestion occurs in the abomasum under the proteolytic action of pepsin and chymosin. Proteinases quickly hydrolyze the Phe^{105}–Met^{106} bond of κ-caseins (Delfour et al., 1965), resulting in clotting. Adding non-casein proteins to the diet modifies proteolysis conditions because protein substitutes do not coagulate in the abomasum (Guilloteau et al., 1975; Ternouth et al., 1975). The absence of curd formation observed after casein was replaced by whey (Pélissier et al., 1983; Toullec et al., 1971), fish (Guilloteau et al., 1975), or soybean (Guilloteau et al., 1979) proteins increases the abomasal emptying of nitrogen compared to that for total milk proteins. Moreover, proteolysis in the abomasum is reduced when 50% of milk proteins are replaced by whey proteins (Caugant et al., 1992).

The nutritional quality of proteins is primarily related to the amino acid composition. However, protein digestibility and amino acid availability also are key factors of protein quality. Studies on gastric digestion, the first step of protein digestion, of various diets containing nonclotting proteins have already been made in preruminant calves (Toullec et al., 1971; Guilloteau et al., 1975, 1979). Such in vivo studies are expensive and time-consuming. Thus, in vitro methods reproducing in vivo proteolysis conditions would be of great interest. Savalle et al. (1989) have developed an in vitro method that simulates within 3 h the in vivo digestion in the abomasum of the preruminant calf normally seen after 6 h.

In a previous experiment (Caugant et al., 1992) gastric emptying of protein fractions of milk replacers containing heated or nonheated whey proteins was studied and correlations between in vivo and in vitro methods of digestion were quantitatively established. Using diets fed in the experiment of Caugant et al. (1992), the purpose of the present work was to characterize the products leaving the stomach and to evaluate the degree of correspondence between the amino acid composition of the digesta reaching the duodenum of calves and that of the digesta leaving the artificial stomach.

MATERIALS AND METHODS

Protein Sources. Three protein sources were studied: skim milk and two whey protein concentrates (WPCs). The WPC was obtained by ultrafiltration and divided in two batches which were heated either at 74° C for 15 s (native WPC) or at 72 °C for 20 min (heated WPC) before spray-drying. The second heat treatment was performed to partially denature the proteins (Paris and Babinsky, 1991).

In Vivo Experimental Procedure. The feeding and collection procedures used were described previously by Caugant et al. (1992). Briefly, three preruminant calves of 45–60 days of age were fitted with a reentrant duodenal cannula (Ivan and Johnston, 1981). After a 7-day postsurgical recovery, they began to receive one of the three experimental diets which differed in protein source: 100% skim milk powder (milk diet); a mixture [50:50 on a crude protein (CP) basis] of skim milk powder and native WPC (WPCN diet) or heated WPC (WPCH diet) (Table I). No emulsifier was added, and the three diets contained 1 g of Ca/100 g of powder. Diets were diluted with water to contain 16% powder. Calves were assigned to a 3 × 3 Latin square design and received each diet for 8 days. On the ninth day, they received in the morning their normal diet with sodium citrate (11 g/kg of powder) added to increase gastric emptying (Frantzen et al., 1973); at the evening meal, the calves received only water to ensure complete emptying of the abomasum. On the following morning, they received their experimental diets and all digesta flowing from the abomasum during 6 h postprandially were collected separately (0–0.5, 0.5–1, 1–2, 2–3, 3–4, and 4–6 h after feeding) in TCA (12% final concentration). Digesta retained were replaced in the upper part of the cannula by digesta collected the week before from the same calves fed their respective diets. Each sample was centrifuged at 10000*g* for 25 min at 5 °C. Supernatants and sediments were stored at −20 °C.

In Vitro Experimental Procedure. Diets used for the in vitro procedure contained no fat premix, lactose, or starch. The three diets contained 2.5% minerals (Union Laitière Normande, Condé sur Vire, France) and 97.5% skim milk or a mixture (50:

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Table I. Composition (Percent) of Milk Replacers (on Powder Basis)

ingredient	diets[a] milk	WPCN	WPCH
fat premix[b]	52.8	52.8	52.8
skim milk powder	27.4		
native whey powder		27.4	
heated whey powder			27.4
lactose	14.3	14.3	14.3
starch	3	3	3
vitamins and minerals[c]	2.5	2.5	2.5

[a] Milk, skim milk powder; WPCN, skim milk powder and native whey protein concentrate (50:50 on a CP basis); WPCH, skim milk powder and heated whey protein concentrate (50:50 on a CP basis). [b] Skim milk powder containing 37% tallow (Nutrinor, Québec, Canada). [c] Nutrinor.

50 on a CP basis) of skim milk and native WPC (WPCN) or heated WPC (WPCH). The milk replacers were reconstituted at 10% powder just before use with deionized water at 40 °C.

The experimental procedure was performed as previously described (Savalle et al., 1989; Caugant et al., 1992). Briefly, 500 mL of the diet was put in an "artificial stomach" and subjected to the action of rennet (520 mg of chymosin, 290 mg of pepsin/L; Boll-Hansen, France). The initial enzyme/substrate ratio was 1/2500 (w/w), and then diluted enzyme (2% liquid rennet) was added at a variable flow rate following an exponential function. A similar function was used to control the digesta emptying flow. Acidification of the medium was controlled and adjusted so that the pH of the medium followed a predetermined curve. This curve was established on the basis of results previously obtained (Scanff et al., 1990) with preruminant calves fed milk. Total effluents were collected separately (20, 30, 60, 90, 120 and 180 min after the beginning of the digestion) in TCA (12% final concentration). Each sample was centrifuged at 10000*g* for 10 min. Supernatants and sediments were stored at −20 °C until analysis.

Chemical Analysis. The sediments (after homogeneization in water) were extracted five times with an equal volume of diethyl ether to eliminate the TCA and then freeze-dried. Each sample was then analyzed by SDS–polyacrylamide gel (SDS–PAGE) electrophoresis according to the procedure of Laemmli (1970) adapted for milk proteins by Trieu-Cuot and Gripon (1981).

Amino acid (AA) composition was determined separately for the TCA-soluble and TCA-insoluble fractions. In vivo samples (0.2 mg of N) were hydrolyzed in vacuum hydrolysis tubes (Pierce Chemical, Rockford, IL) at 105 °C for 24 h in 6 N HCl using norleucine (10 μmol/mL) as the internal standard. After hydrolysis, samples were suspended in a buffer (pH 2.2, Beckman, Palo Alto, CA) and filtered through 0.2-μm millipore filters (Minisart NML, Sartorius, Goettingen, Germany). Determination of AA was carried out by ion-exchange chromatography using a Beckman amino acid analyzer (System 6300). Hydrolysis conditions for digesta obtained from the in vitro procedure were slightly different (110 °C, HCl 5.7 N), and a Biotronik analyzer (LC 5000, Munich, Germany) was used.

Statistical Analysis. The AA compositions of digesta obtained in vivo and in vitro were submitted to a factorial correspondence analysis (Guilloteau et al., 1983). This method considers each sample as a point in a 16-dimensional space with 16 variables (the percent of each of the 16 AA assayed). The initial pattern is reduced to a bidimensional space in which the two axes are not correlated. As the distance between two points characterizing two samples decreases, similarity between the two samples increases.

Percentage of casein and whey or WPC in the digesta obtained in vivo and in vitro was assessed by iterative calculation of the theoretical mixture which minimized the χ^2 distance with respect to the observed AA composition of the digesta (Duvaux et al., 1990). Reference values of AA composition of casein, whey, and WPC were obtained from AA analysis of the casein and whey fractions obtained by precipitation at pH 4.6 of skim milk and of the WPC, respectively. As the χ^2 distance decreases, similarity between the protein mixtures increases.

RESULTS

All calves remained in good health throughout the experiment without fever, diarrhea, or other apparent sickness. The cannulae remained functional during the trial.

Electrophoretic Analysis of the Products Leaving the Stomach. The electrophoretic pattern of the milk diet and the digestion products obtained in vivo are shown in Figure 1(A1). Bands corresponding to the main caseins (α_{s1}, β) were present in small amounts in digesta flowing from the abomasum during the first hour, but the proportion of casein in these digesta samples was much lower than in the milk diet. After 1–2 h of digestion, peptides (MW < 10 000) were detected. One calf had a high proportion of casein and casein degradation products in the digesta flowing between 0 and 0.5 h (results not shown). Caseins were present in the digesta obtained with the WPCN diet [Figure 1(A2)] for 2 h after feeding (but in lower proportion than in the WPCN diet), and products of degradation started to appear thereafter. With the WPCH diet [Figure 1(A3)], caseins disappeared a little later. Casein proportions in the first two samples were higher than with the WPCN diet. α-Lactalbumin flowed from the abomasum for 3 h postfeeding with the milk and WPCN diets; this whey protein was present for 6 h in digesta obtained with the WPCH diet. β-Lactoglobulin was still present in large amounts in all digesta samples between 0 and 6 h with the three diets.

The electrophoretic patterns of digesta collected in vitro [Figure 1 (B1–B3)] were similar to those of digesta obtained in vivo. However, the protein emptying rate was stimulated 2-fold in vitro compared with that in vivo. With the milk diet, caseins disappeared rapidly (20 min after the beginning of digestion). With diets containing whey proteins, caseins disappeared later (after 30 and 60 min for diets WPCN and WPCH, respectively). α-Lactalbumin and β-lactoglobulin were almost completely present in all samples, with a small decrease in the amount of α-lactalbumin at the end of the digestion, especially with the milk diet.

Amino Acid Composition of the Gastric Effluents. *Multivariate Analysis.* Multivariate analysis by factorial correspondence analysis was performed with all samples obtained in vivo and in vitro (not shown). Because of the large number of points which are superimposed, interpretation of such a representation is somewhat difficult. Thus, to visualize the evolution of the AA composition of the samples, comparisons by multivariate analysis (Figure 2) of the in vivo and in vitro experiment (parts A and B, respectively) were conducted separately. When all in vivo or in vitro digesta were considered, two main groups could be constituted: one corresponding to protein products (insoluble in 12% TCA) and the other to small peptides (soluble in 12% TCA). The three diets and references proteins (whey, casein, and WPC used in this experiment) were introduced in the analysis.

In vivo, the AA composition of the TCA-insoluble fraction was located between that of casein and that of whey proteins (Figure 2A). With the milk diet, sediment composition appeared more like the composition of milk and casein as postfeeding time increased. The digesta sediment composition of the WPCN and WPCH diets was located between the composition of diets and whey but closer to the diet composition. Changes in sediment composition were more important with time in vitro than in vivo (Figure 2B), especially with the milk diet. The sediment composition of in vitro digesta after feeding the milk diet was close to that of the diet during the first

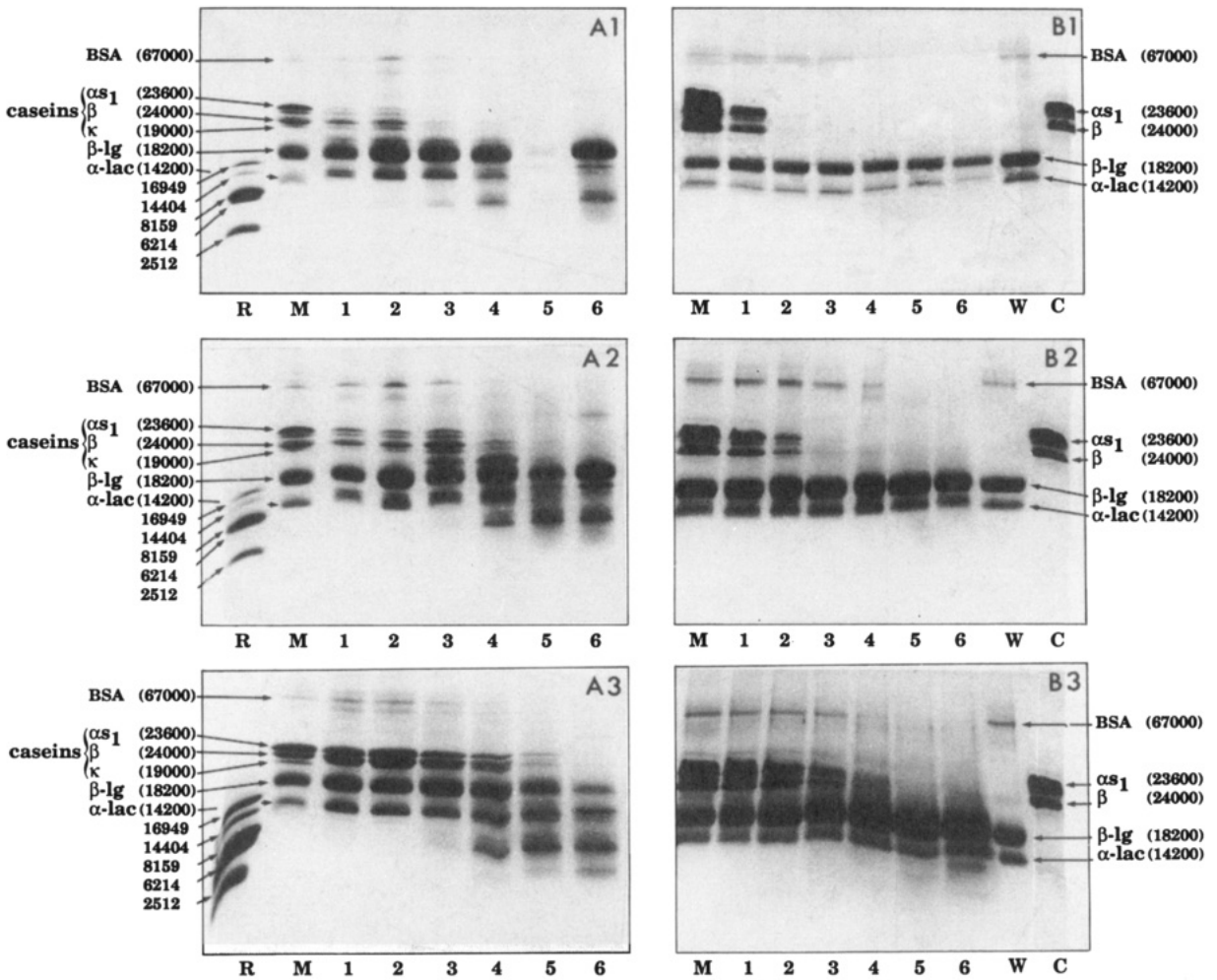

Figure 1. SDS electrophoresis of 12% TCA insoluble fractions of samples leaving the abomasum of the calves (A) and the artificial stomach (B) with the diets milk (1), WPCN (2), and WPCH (3). R, reference molecular weight; M, meal; W, whey proteins; C, casein. Numbers represent times after the beginning of the digestion (in vivo, in vitro): 1, 0–0.5, 0–0.33 h; 2, 0.5–1, 0.33–0.5 h; 3, 1–2, 0.5–1 h; 4, 2–3, 1–1.5 h; 5, 3–4, 1.5–2 h; 6, 4–6, 2–3 h.

0.5 h. During the next 60 min (0.5–1 h and 1–1.5 h) sediment composition looked like that of whey before becoming closer to the casein composition thereafter. For the WPCN and WPCH diets, evolution of AA composition of the digesta was similar to that observed in vivo.

The changes in the AA composition of the supernatants were more pronounced than those in the AA composition of the sediments. In vivo, the supernatants obtained after feeding the milk diet were situated in between CMP and casein in the first hour of digestion and thereafter moved toward casein and milk diet. With the WPCN and WPCH diets, supernatant composition was closer to that of CMP during the first hour. Thereafter, supernatant composition moved toward an area situated among the casein and the WPCN and WPCH diets. Postfeeding changes in AA composition were similar in vitro and in vivo. However, differences in the milk diet digesta and the digesta from the other diets were more important in vitro. At the end of the collection (2–3 h), AA composition of the in vitro digesta obtained with the WPCN and WPCH diets was farther from the diets than in vivo.

Emptying of Casein, Whey, and WPC Proteins in the Protein Fraction of the Gastric Effluents. The proportions of casein, whey, and WPC proteins in the TCA-insoluble fraction of samples was assessed by minimizing the χ^2 distance between the AA composition of the samples and that of a mixture of these components. For the samples obtained with the milk diet, χ^2 distances were calculated between the AA composition of the samples and a mixture of casein and whey proteins. For the samples obtained with the diets containing WPC, a mixture of casein and WPC was used for the χ^2 calculation. The protein composition of the milk diet, as determined from its AA composition, was close ($\chi^2 = 5$) to that of a mixture of 81% casein and 19% whey. The AA compositions of the WPCN and WPCH diets were similar to that of a mixture of 46% casein and 54% WPC ($\chi^2 = 7$ and 5, respectively).

In vivo (Figure 3A), the proportion of casein in the digesta decreased during the first 30 min after feeding the milk diet (37%, $\chi^2 = 14$), increased thereafter to reach 70% ($\chi^2 = 8$) 4 h postfeeding, and decreased during the last 2 h to reach 56% ($\chi^2 = 8$). One calf fed the milk diet had a high proportion of casein in the digesta obtained 30 min after feeding (71% against 37 and 34% for the other two calves). Because of this strange behavior, AA compositions for the in vivo digesta obtained with the milk diet were calculated without this calf. With the WPCN diet, the proportion of casein decreased to reach 33% during the first hour of digestion ($\chi^2 = 12$) and did not change greatly thereafter: 49% after 2 h and 53% after 6 h ($\chi^2 = 13$ and 17, respectively). With the WPCH diet, the proportion of casein remained constant with time after feeding (51% after 30 min and 47% after 6 h, $\chi^2 = 9$ and 20, respectively).

Protein composition of digesta obtained from the in vitro procedure (Figure 3B) showed that the AA composition of the first sample obtained with the milk diet was

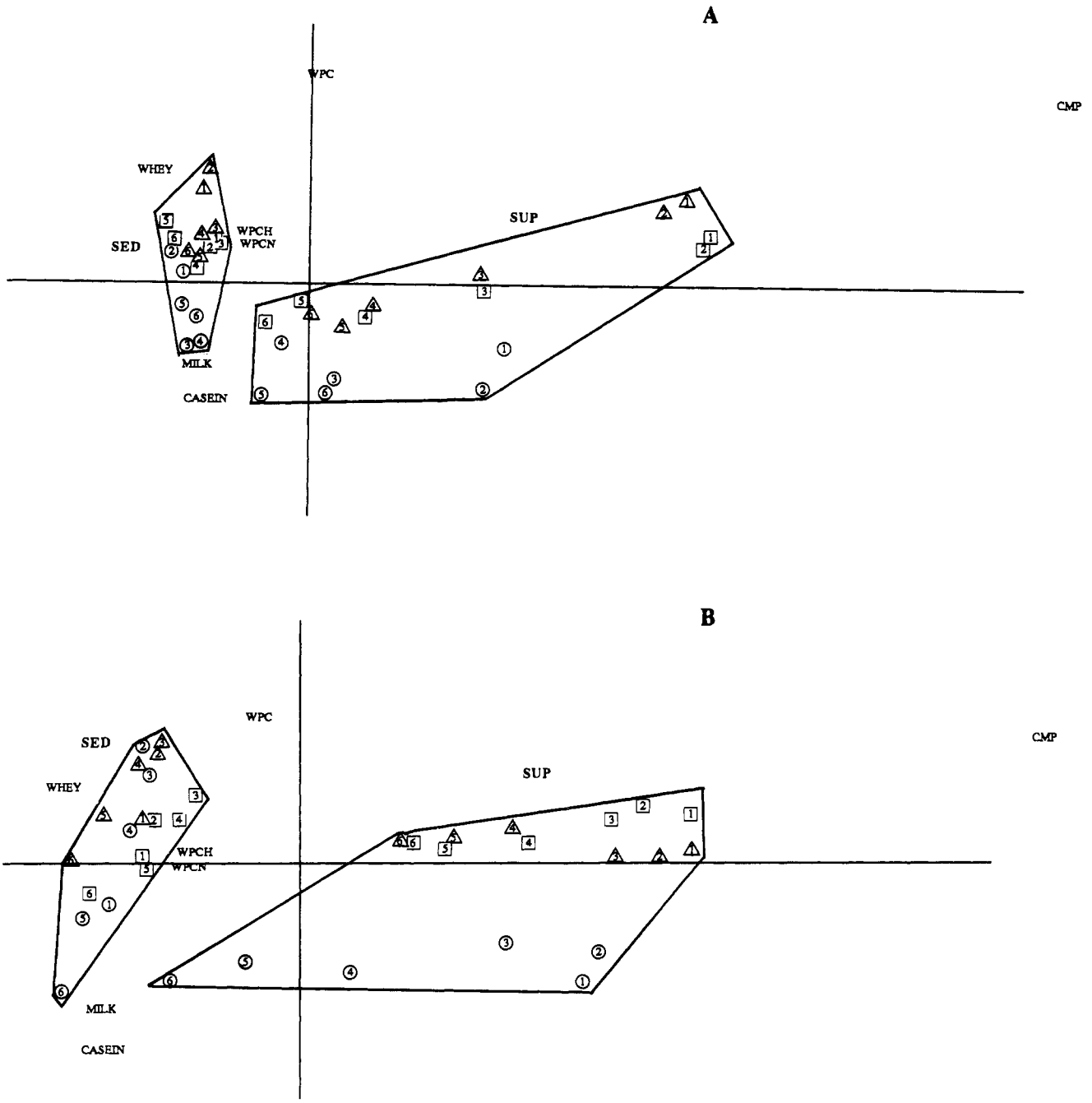

Figure 2. Factorial correspondence analysis performed on the relative quantities of the amino acids (percent of the sum of assayed amino acids) on each sample leaving the abomasum of the calves (A) and the artificial stomach (B) with the diets milk (○), WPCN (△), and WPCH (□). Numbers represent times after the beginning of the digestion (in vivo, in vitro): 1, 0–0.5, 0–0.33 h; 2, 0.5–1, 0.33–0.5 h; 3, 1–2, 0.5–1 h; 4, 2–3, 1–1.5 h; 5, 3–4, 1.5–2 h; 6, 4–6, 2–3 h. CMP, caseinomacropeptide; SUP, supernatants; SED, sediments.

similar to a theoretical mixture of 56% casein and 44% whey ($\chi^2 = 9$). Then, the amount of casein decreased (11%). From 60 min postfeeding, the proportion of casein increased and reached 69% at 3 h ($\chi^2 = 12$). With the WPCN diet, the proportion of casein was 36% ($\chi^2 = 5$) in the first sample and decreased until 60 min (14%, $\chi^2 = 7$). It increased thereafter to reach 49% casein at 180 min ($\chi^2 = 13$). Changes in the proportion of casein and whey in the digesta obtained with WPCH were smaller than those obtained with the WPCN diet. Casein proportion in the first sample was 49 ($\chi^2 = 6$) and 31% ($\chi^2 = 11$) at 60 min and 61% ($\chi^2 = 11$) at 180 min.

Emptying of Some Characteristic Amino Acids (Determined after Acid Hydrolysis of Gastric Effluents). Digesta AA composition changed after feeding with the effect being more important for proline (in the TCA-insoluble fraction) and threonine (in the TCA-soluble fraction).

The initial percentages of proline in the sediments of the milk, WPCN, and WPCH diets were 10.9, 9.0, and 8.9, respectively (Figure 4). With the milk diet, the percentage of proline in the TCA-insoluble fraction of digesta decreased sharply 30 min (in vitro) or 1 h (in vivo) after the beginning of digestion (decreases of 36 and 38% of the initial value in vivo and in vitro, respectively). Thereafter, it increased gradually, with the increase being more important in vitro than in vivo. With diets containing WPC, the decrease in the percentage of proline during the first 30 min of digestion was not as important as it was for the milk diet: 28 and 15% in vivo and 17 and 7% in vitro for WPCN and WPCH, respectively.

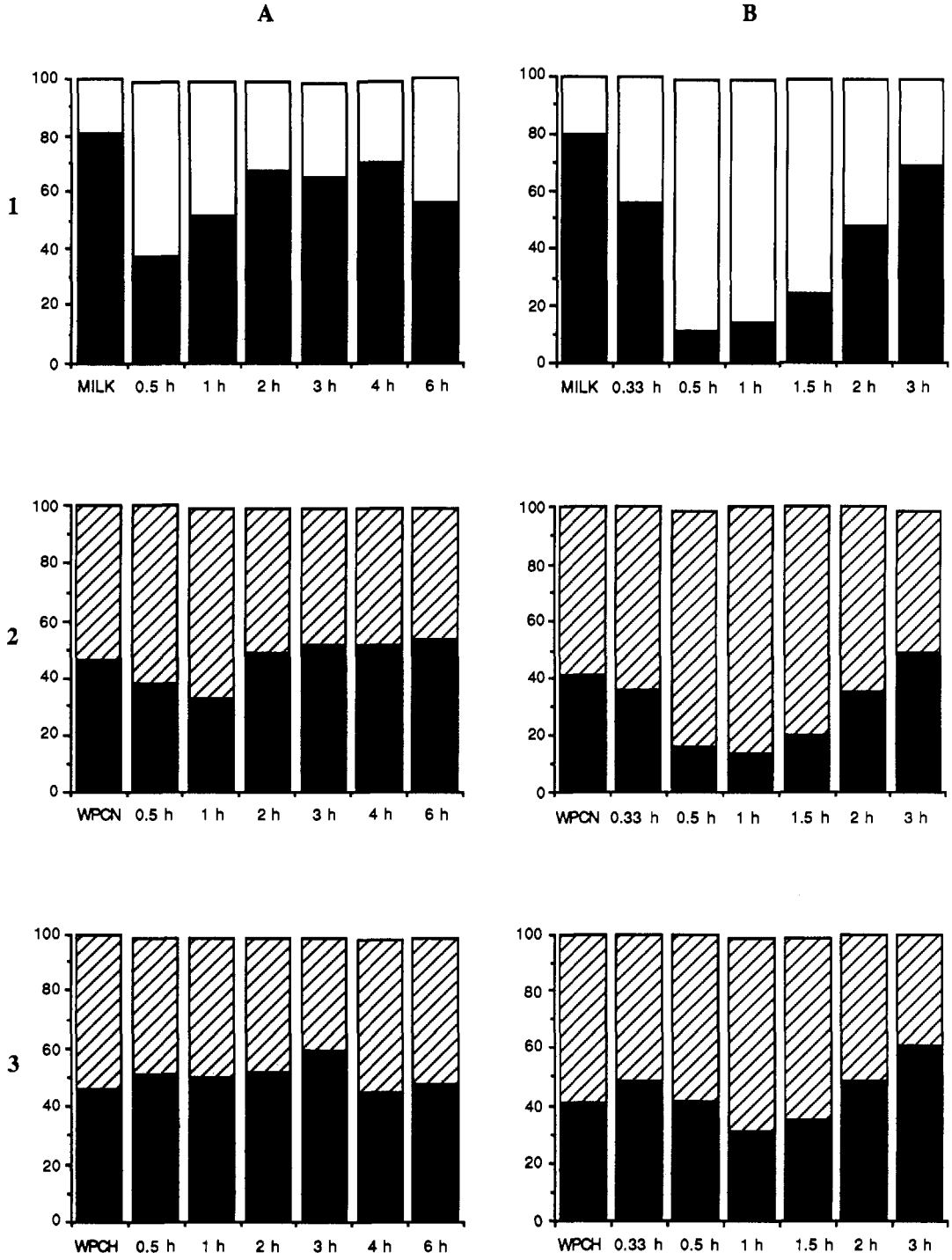

Figure 3. Percentage of casein (■), whey (□), and WPC (▨) in the TCA-insoluble fractions of the digesta leaving the abomasum of the calves (A) and the artificial stomach (B) with the diets milk (1), WPCN (2), and WPCH (3).

The initial percentages of threonine in the supernatants of the milk, WPCN and WPCH diets were 6.6, 11.5, and 11.8, respectively (Figure 5). The percentage of threonine in the TCA-soluble fraction of the digesta obtained with the milk diet increased sharply after the meal (decreases of 23 and 42% after 1 h in vivo and 30 min in vitro, respectively) and decreased thereafter. With the WPCN and WPCH diets, the percentage of threonine decreased slowly after feeding, with the decrease being more pronounced in vivo.

DISCUSSION

Coagulation of the milk diet occurred during the first 30 and 20 min for the in vivo and in vitro experiments, respectively, as shown by the rate of disappearance of caseins in the electrophoretic analysis. For both in vivo and in vitro digesta, the AA composition of the products flowing from the abomasum after feeding looked more like the AA composition of whey proteins than that of casein, which is in agreement with the results of Yvon et al. (1985). Proportions of whey and casein in digesta as estimated from the AA composition of the diet changed rapidly after feeding. Proline percentage decrease in sediments also shows that casein is trapped in the clot. Casein contains twice as much proline (10.5%) as whey proteins (5.1%) (Yvon et al., 1985); therefore, changes in proline percentage of sediments could reflect the importance of coagulation. In fact, coagulation results in casein

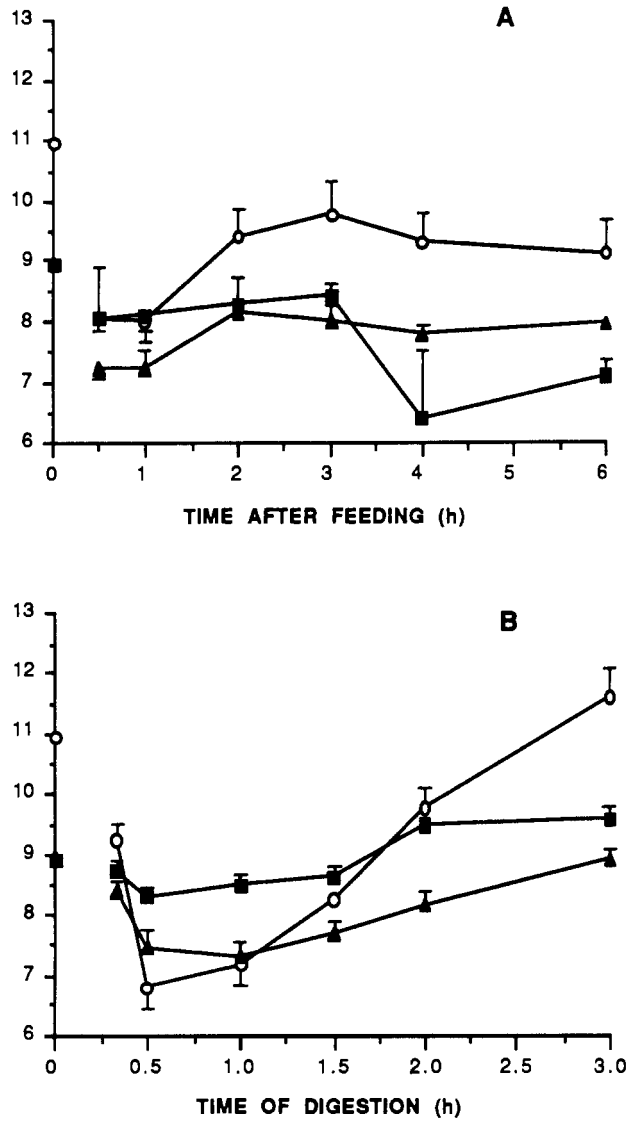

Figure 4. Percentage of proline in the TCA-insoluble fractions of the digesta obtained in vivo (A) and in vitro (B) with the diets milk (○), WPCN (▲), and WPCH (■). Values at $t = 0$ are that of the TCA-insoluble fractions of the diets.

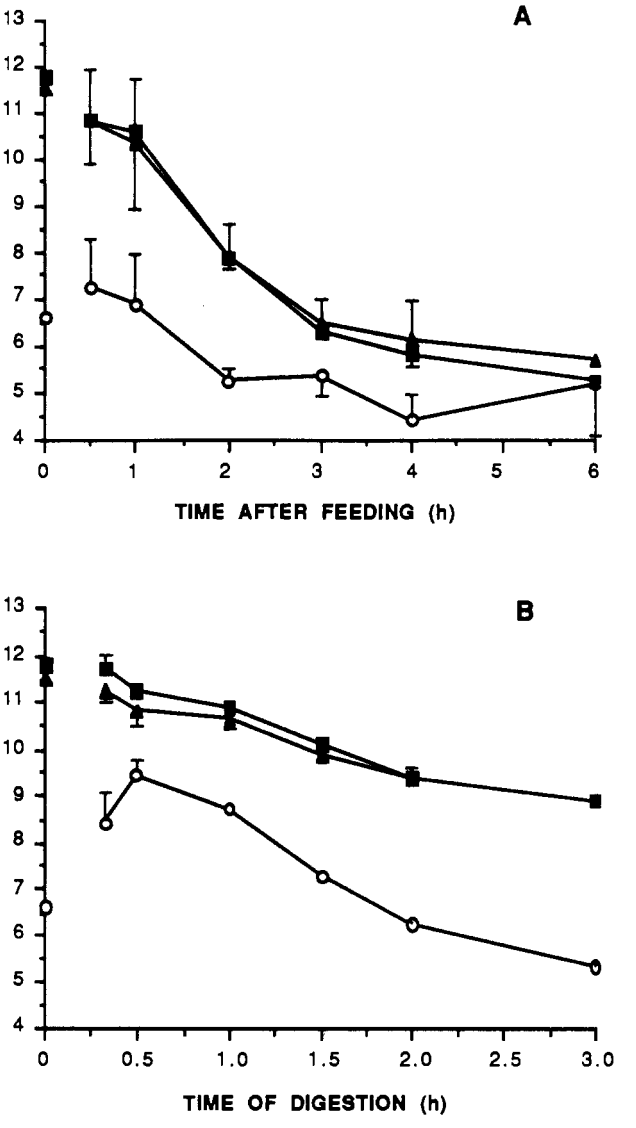

Figure 5. Percentage of threonine in the TCA-soluble fractions of the digesta obtained in vivo (A) and in vitro (B) with the diets milk (○), WPCN (▲), and WPCH (■). Values at $t = 0$ are that of the TCA-soluble fractions of the diets.

being trapped in a clot in the abomasum of preruminant calves (Petit et al., 1987). The proportion of casein coagulating may be estimated from gastric emptying of digesta (Caugant et al., 1992) and casein proportion in digesta; during the first 30 min of in vitro digestion, 94% of ingested casein had coagulated and 92% during the first hour of in vivo digestion.

Gastric emptying of casein during the first sampling time of digestion seemed to be more pronounced in vivo than in vitro. However, one calf fed the milk diet had a particular behavior; the electrophoresis analysis showed peptides from casein hydrolysis as early as the first 30 min of gastric digestion. The proportion of casein in digesta, as estimated from the AA composition, was very high for this calf, and this could not result from the morning meal. This calf was probably not totally fasted before the collection procedure started.

Differences in the proportion of casein being released in vivo and in vitro could be explained by the different diets used in this experiment. In vivo, diets formulated for the calves contained components (starch, lactose, tallow) not included in the diets used for the in vitro procedure. These components might modify the structure of the casein micelles in the diet (Mozersky et al., 1991) as well as that of the coagulum obtained in vivo and the coagulation phenomenon since the same casein emptying was observed in vitro and in vivo with calves fed milk (without other nutrients) (Yvon et al., 1992). Finally, because of peristalsis in the abomasum and the motility of the pylorus and the duodenum, casein could be released more rapidly in vivo than in vitro.

The first peptide originating from casein hydrolysis is CMP, which is known to be released rapidly after feeding (Yvon and Pélissier, 1987). Caseinomacropeptide contains more threonine (16.7%) than both casein (4.4%) and whey protein (5.2%) (Yvon et al., 1985, and personnal communication), and the glycosylated fraction of CMP is soluble in 12% TCA. Therefore, changes in threonine content of supernatants (TCA-soluble fractions) could reflect the gastric emptying of CMP. The presence of CMP in the first sample after feeding was corroborated by the factorial correspondence analysis: supernatants of digesta obtained with the milk diet were found between CMP and casein.

After some hours, the electrophoresis analysis allowed detection of many large peptides, insoluble in 12% TCA, corresponding to the hydrolysis of casein. The origin of these peptides was confirmed by estimating the proportion

of casein (or peptides originating from casein) in the TCA-insoluble fraction of the digesta which increased from 11 (at 30 min) to 69% (at 180 min) in vitro, and from 37 (at 30 min) to 70% (at 4 h) in vivo. The increasing amounts of peptides in the supernatant explained the evolution of the AA composition of the supernatants toward the casein during digestion. Gastric emptying of casein was delayed as a result of coagulation and most casein ingested was released from the stomach as peptides. Similar results have been reported by Scanff et al. (1990, 1991).

Whey proteins (α-lactalbumin and β-lactoglobulin) were emptyed with the liquid fraction. Proteins of whey were the main components of the first digesta collected after feeding. The β-lactoglobulin was present in all samples, which corroborates that this protein is not hydrolyzed by gastric enzymes as shown by Yvon et al. (1984). Reddy et al. (1988) showed that β-lactoglobulin is resistant to peptic and chymotryptic digestion because of its stable conformation. No particular observation could be made concerning the emptying of α-lactalbumin.

With the WPCN and WPCH diets, the coagulation phenomenon was less important and it occurred later than with the milk diet. Caseins were detected in the electrophoretic pattern of digesta sediments later postfeeding compared to the milk diet. Coagulation occurred 30 min (in vitro) and 1 h (in vivo) after the beginning of digestion with the WPCN diet. Feeding WPCH delayed coagulation which occurred 60 min (in vitro) and 2 h (in vivo) postfeeding. In vitro, the proportions of casein coagulating were estimated to be 86 to 79% after 30 min for the WPCN and WPCH diets, respectively. The proportion of casein being trapped in the coagulum was lower when whey proteins were incorporated in the diet compared to that for a diet with only milk protein. Coagulation seemed to be less important in vivo than in vitro with 73 and 55% of the casein being coagulated after 1 h with the WPCN and WPCH diets, respectively. The reduction in percentage of proline was smaller with the WPCN and WPCH diets than with the milk diet, which shows that caseins were still present in the digesta at the beginning of the digestion. However, digesta contained the CMP fraction and diets contained CMP as a result of whey originating from rennet coagulation. As CMP has a high proline content (10.8%), changes in proline percentage of digesta could not be solely representative of the gastric emptying of casein.

In the first hours of digestion, digesta supernatants obtained from calves fed diets containing whey proteins contained mainly the soluble fraction of CMP, which is the glycosylated fraction (Yvon and Pélissier, 1987). As a result, AA composition of these samples appeared to be closer to that of CMP than to that of the others as determined with the factorial correspondence analysis. At the same time, gastric emptying of threonine was very high, which shows that some CMP is released in the TCA-soluble fraction of digesta. In the last part of digestion, peptides released from casein hydrolysis reached the duodenum and AA composition of TCA-soluble and -insoluble fractions of digesta were approaching that of casein. In agreement with results observed with the milk diet, dietary whey proteins (α-lactalbumin and β-lactoglobulin) were released with the liquid fraction of the diet.

Previously, Tagari and Roy (1969) and Ternouth et al. (1974) found that heating spray-dried skim milk powder at 74° C for 30 min induced decreased proteolysis in the abomasum. Heating milk proteins induced interactions between β-lactoglobulin and caseins, which decreased coagulability of milk and altered proteolysis (Scanff et al., 1990, 1992). In the present experiment, heat treatment only concerned the whey protein concentrate. Coagulation seemed to occur somewhat later for the WPCH than for the WPCN diet, but there was no difference in digestion products or in AA composition of the digesta. This suggests that moderate heat treatment does not significantly affect proteolysis in the abomasum or in the in vitro stomach. This was previously observed concerning the emptying of nitrogen fractions of similar diets (Caugant et al., 1992).

The in vitro method was developed on the basis of results obtained with preruminant calves fitted with reentrant duodenal cannula. This artificial stomach satisfactorily reproduced gastric digestion of protein measured in these animals. However, to what extent can a cannulated calf be representative of an intact animal? Bueno et al. (1981) reported that in the fasted dog retrograde flow from the duodenum to the antrum occurred during the development of a migratory motor complex, especially during the quiescence phase. This leads to elevation of the antral pH to about 5 pH units and retrograde flow of bile salts. If the same phenomenon occurred in the fasted calf, it would be the same whatever the diet and could have occurred with a cannula or not because the cannula was closed before collection; the transit was also normal. After feeding, gastric emptying of large amounts of liquid diet will probably prevent this retrograde flux. Moreover, it has been shown that the flow of digesta from the abomasum is controlled by the composition of material entering the duodenum (Smith and Sissons, 1975). The surgical procedure for inserting a cannula into the duodenum of the calf may affect the regulation of secretory processes and motility of the abomasum in comparison with those of an intact animal. However, the reentrant cannulation method used in the present experiment did not require intestinal transection and little damage occurred to the blood and nervous system. Also, the cannula may physically restrict or change the course of digesta flow, because peristaltic contractions failed to propagate beyond the reentrant cannula (Wenham and Wyburn, 1980). Nevertheless, Sissons and Smith (1982) observed that inserting a reentrant cannula in the proximal duodenum of the preruminant calf did not lead to major disturbances in net abomasal secretion and digesta flow to the duodenum.

CONCLUSION

When preruminant calves were fed a milk diet, ingested caseins were mainly (92–94%) retained in a clot and released progressively from the stomach as peptides. With milk replacers containing whey proteins mixed (50:50 on a CP basis) with milk proteins, the coagulation phenomenon in the abomasum was delayed and the clot obtained was less effective in restraining casein emptying. Gastric emptying of protein nitrogen and total nitrogen have been shown to increase (Caugant et al., 1992) with the WPCN and WPCH diets compared with that for the milk diet. In this study, it was also observed that gastric emptying of CMP during the first hour after feeding was high. Results obtained with both in vivo and in vitro methods were generally similar. This confirms that this technique could be used for measurements of milk protein digestion in the abomasum of preruminant calves. Furthermore, the use of the in vitro stomach to study the gastric digestion of milk replacers allowed a faster, easier, and cheaper determination than in vivo assays.

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