Effect of Some Technological Treatments of Milk on Amino Acid Composition of in Vivo Effluents during Gastric Digestion

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The effects of technological treatments of milk on the in vivo amino acid (either in the peptidic or in the proteic form) gastric emptying of preruminant calves were studied with three different diets: raw skim milk (RSM), pasteurized skim milk (PSM), and pasteurized and acidified skim milk (Y). The emptying of casein and whey proteins was evaluated from the amino acid compositions obtained by acid hydrolysis of the effluents leaving the stomach during 12 h. From these compositions, the emptying kinetics of each amino acid, essential and hydrophobic amino acids, were also considered. It was found that casein was emptied as was the fresh matter with Y and retained longer in the stomach with RSM and PSM because of its coagulation. Since interactions occurred during heating between casein and whey proteins, whey proteins were emptied as was the fresh matter with Y and RSM, whereas they were also retained with PSM. Gastric emptying kinetics of some amino acids (Asx, Pro, Leu, Thr, Tyr, Phe) were significantly affected by milk process. Consequently, the pool of amino acids entering the duodenum varies with time and diet also. Consequences of these variations are discussed.

INTRODUCTION

The composition of the bioavailable pool of amino acids is of prime importance for the utilization of these amino acids by the organism. Some researchers have reported the favorable effect of branched-chain amino acids on nitrogen metabolism as well as the effect of the essential amino acid/total amino acid ratio on the proteic efficiency of an amino acid mixture. If the optimal essential/total amino acid ratio or the proportion of each essential amino acid in the total of essential amino acids is improper, ingested amino acids, which can quantitatively fulfill requirements, must be spontaneously catabolized (Ikemoto et al., 1989) and be responsible for troubles. Others researchers have demonstrated that a high level of acetate-generating amino acids in an amino acid mixture increased the plasma cholesterol level, especially in the LDL and VLDL fractions, which increases risk for coronary disease (Alladi and Shanmugasundaram, 1989).

The composition of the pool of amino acids arriving at the intestine does not depend only on the dietary protein composition. In fact, Huff et al. (1977) demonstrated that feeding a mixture of amino acids corresponding to casein produced an elevation of plasma cholesterol similar to that obtained with casein itself, whereas feeding a mixture of amino acids corresponding to soy protein gave a higher plasma cholesterol level than intact soy protein. It depends also largely on the digestion process. In fact, after feeding, proteins are submitted to the digestion process and the composition of the amino acid pool appearing in the blood is the result of this process and also of the enterocyte absorption and transport. The first step of protein digestion is gastric digestion. Yvon and Pélissier (1987) showed that the stomach regulates the release of proteins and selects proteins and peptides that enter the gut. This was shown on calves fed skim milk. These authors showed a progressive and sequential evacuation of peptides into the duodenum. Thus, the stomach has a major effect on the amino acid composition of digesta that enter the gut.

Many studies on gastric digestion of milk reveal that technological treatments modify its digestion (Kaufmann, 1984; Meisel and Hagemeister, 1984; Miranda and Pélissier, 1987; Scanff et al., 1990). They modify the coagulation that occurs in the stomach, and the structure of the coagulum influences the nature of the products leaving the stomach. In this study, we describe for the first time how some technological treatments of milk can influence the pool of amino acids (either in the peptidic or in the proteic form) that is sequentially emptied from the stomach into the gut. For that, we compare the amino acid gastric emptying of a reference diet, i.e., bovine raw skim milk (RSM), and the amino acid gastric emptying of the same milk that was pasteurized (PSM) or pasteurized and acidified (Y). This study was made with preruminant calf, which is a model of monogastric animals.

MATERIALS AND METHODS

Diets. Three types of test meals were prepared from the same skim milk: raw skim milk (RSM), pasteurized skim milk (PSM), and pasteurized and acidified skim milk (Y). To obtain diets PSM and Y, milk was heated at 95 °C for 45 s. For diet Y, the milk was then inoculated (3%) with a preculture of mixed strains, *Streptococcus thermophilus* (CNRZ TJ160) and *Lactobacillus bulgaricus* (CNRZ 1B369), on milk, and then incubated at 42 °C during 4 h. The samples were brought to 4 °C and kept at that temperature for 15 days. Polyethylene glycol (PEG) 4000 was added to each diet to a final concentration of 1% to estimate the ratio of endogenous secretions in the emptied fresh matter.

Animals. Two preruminant Friesian calves were used. At about 1 month of age, each animal was fitted with a reentrant duodenal cannula and an abomasal cannula. Proximal and distal parts of the duodenal cannula were located around 8 and 15 cm after the pylorus, respectively (Ash, 1962). Animals were allowed to recover from surgery for 7 days before digesta collection was started. Their normal diet was a milk substitute based on skim-milk powder, whey powder, and tallow.

Experimental Procedure. For each experiment, two animals were used simultaneously. The day before experiments the animals received in the morning their normal diet to which sodium citrate (11 g/kg) had been added to accelerate gastric emptying (Frantzen et al., 1973) and in the evening only water. Under these conditions, the abomasum was empty at the moment of the first meal in the morning of the experimental days.

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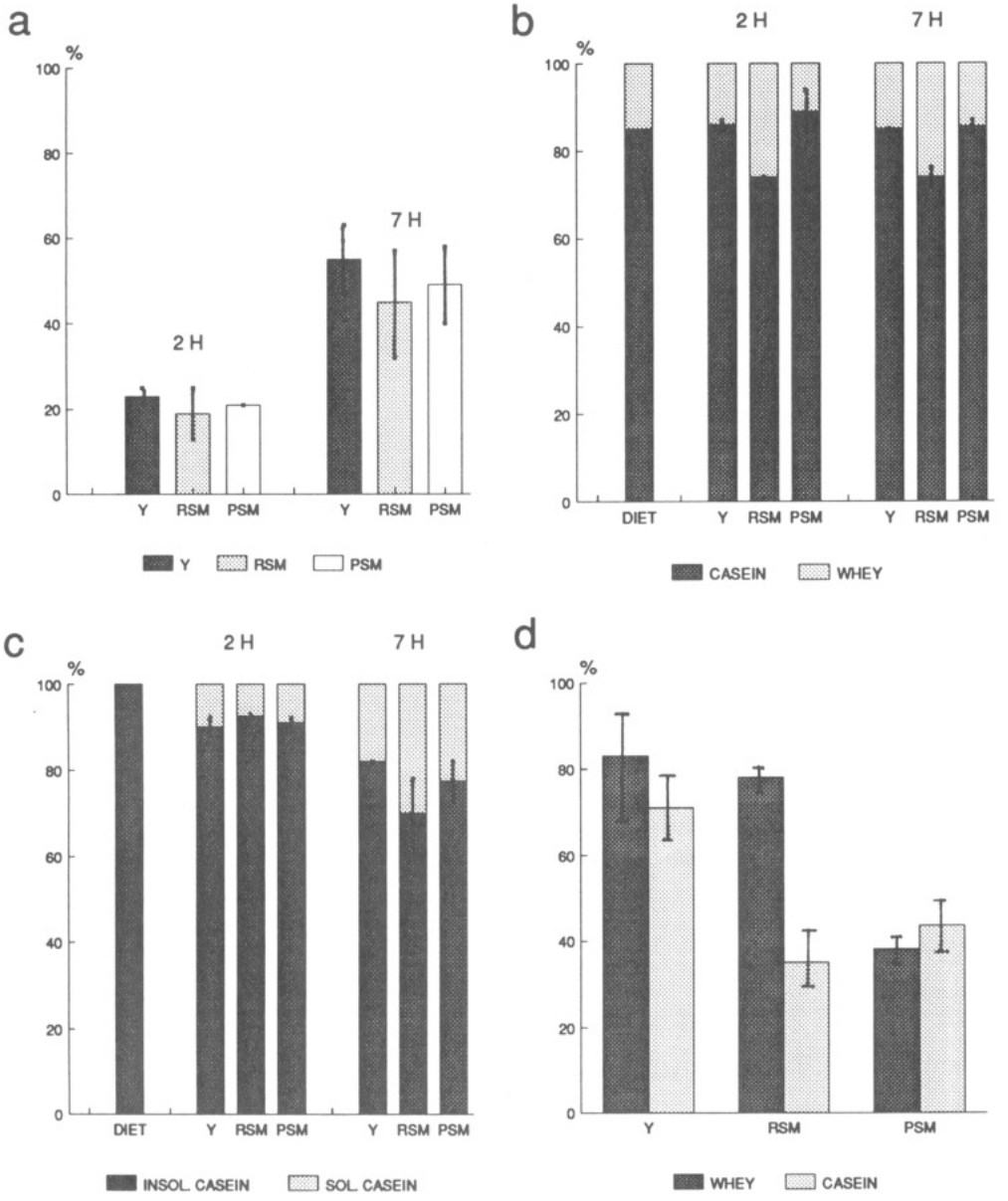

Figure 1. Gastric emptying of proteic matter for the three diets. (a) Total emptied proteins 2 or 7 h after the first meal in relation to the ingested one. (b) Proportion of whey proteins and casein in the emptied proteins 2 or 7 h after the first meal. (c) Proportion of insoluble casein and soluble casein in the emptied casein fraction 2 or 7 h after the first meal. (d) Emptied whey proteins or casein during the 12 h in relation to the emptied fresh matter.

In the morning of the experiment, the two calves recieved a test meal (190 g/kg of live weight$^{0.75}$) of the same diet which was instilled into the abomasum with a peristaltic pump. The duration of the instillation was between 3 and 9 min. The whole effluent from the stomach of the first calf (experimental) was then collected over 7 h, fractions being taken during 10-min intervals for the first 30 min, 15-min intervals for the second 30 min, and 30-min intervals until the end of the 7-h period. A second identical test meal was then given, and samples were collected with the same periodicity up to 5 h after this meal. The digesta coming from the second calf (reference) were instilled in the distal part of the cannula of the first calf at its gastric emptying velocity starting as soon as digesta appeared in the proximal part. A milk hydrolysate was instilled in the duodenum of the second calf, as reported by Pélissier et al. (1983). Each calf received, once a week, the three experimental diets successively. With this procedure, each animal was its own reference.

Aliquots were used to measure pH and then returned to samples. Each sample was immediately precipitated with trichloroacetic acid (TCA) to a final concentration of 12%. Samples were centrifuged at 2000*g* for 20 min. The pellet was resuspended in water. Supernatants and sediments were kept at −20 °C until analysis.

Analysis. For each sample, amino acid composition was determined on the proteic fraction (PN, 12% TCA insoluble fraction) and on the peptidic fraction (NPN, 12% TCA soluble fraction) after acid hydrolysis (5.7 N HCl, 110 °C, 24 h, under vacuum) by using the method of Spackman et al. (1958) with a Biotronik LC 5000 analyzer.

Calculations. From the amino acid compositions, the proportion of the different proteins which could be the main constituents of digesta was assessed by iterative calculation of the theoretical mixture which minimized the χ^2 with respect to the observed composition of digesta. χ^2 was computed as (Vessereau, 1987)

$$\chi^2 = \sum_{k=1}^{17} \frac{(AA_{ik} - AA_{jk})^2}{AA_{jk}}$$

where AA_{ik} and AA_{jk} are the respective percentages of AA_k in the sum of the assayed amino acid in the digesta i and the theoretical mixture j; k, which represents the different amino acids, varies between 1 and 17. The smaller the χ^2, the more minor the difference between the compared mixture. This approach has already been used by Guilloteau et al. (1980, 1986). With this method, the quantities of whey proteins and casein emptied at each digestion time were determined. The same calculation on PN was used to determine casein quantities in PN. The difference between the casein quantity in whole digesta and the casein quantity in PN gives the casein quantity in NPN.

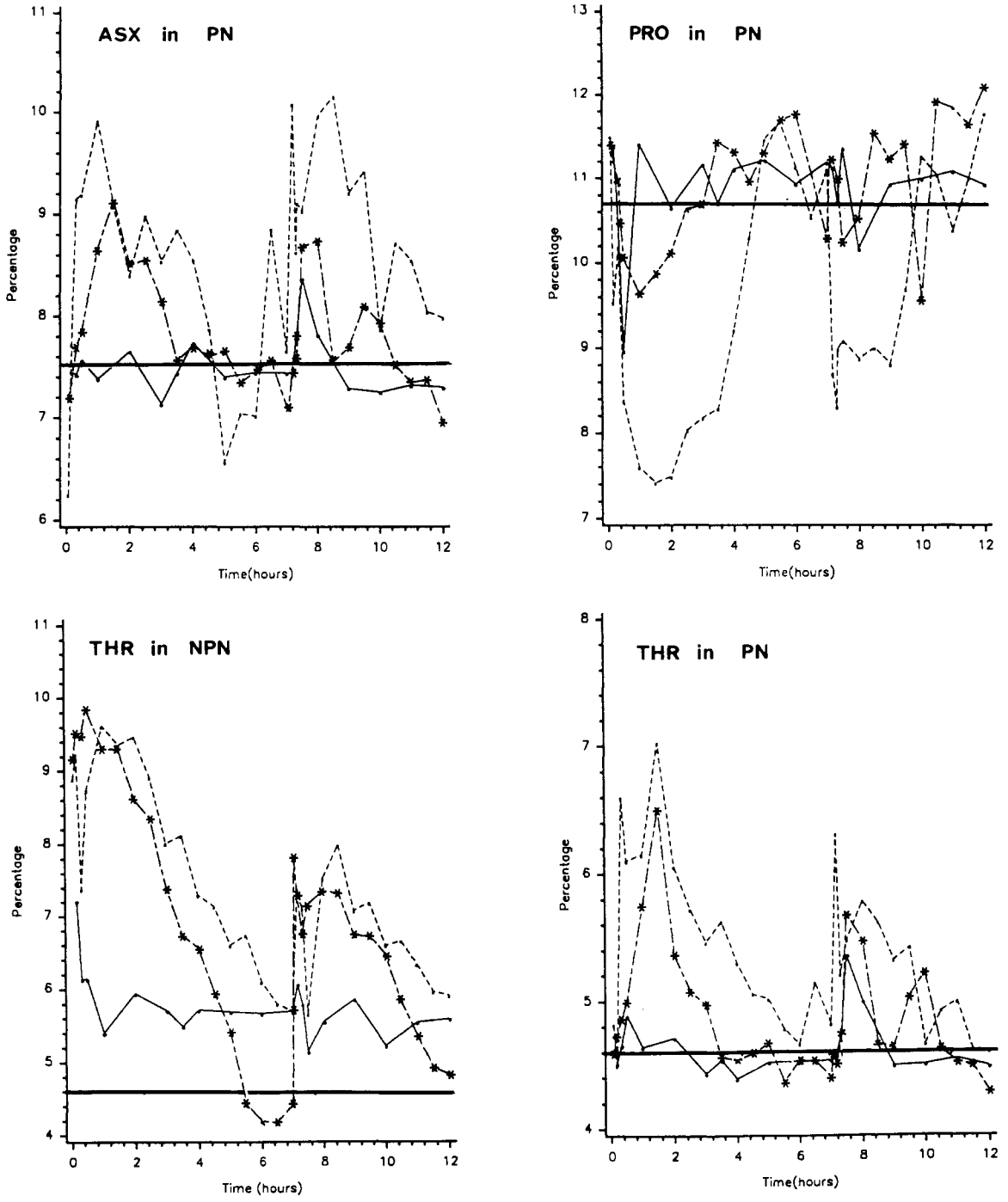

Figure 2. Change with digestion time of amino acid level in digesta (in percentage of the total amino acids). (—) Y; (- - -) RSM; (*) PSM; (—) level in milk.

The protein nitrogen and amino acids digestibilities and a relative digestibility index were calculated for all digesta as follows:

protein nitrogen digestibility (%) = A

$$A = \frac{\text{total quantity of all amino acids in the sample (g)}}{\text{total quantity of all amino acids in the ingested diet (g)}}$$

amino acid digestibility (%) = B

$$B = \frac{\text{total quantity of one amino acid in the sample (g)}}{\text{total quantity of the same amino acid in the ingested diet (g)}}$$

relative digestibility index (RDI) = B/A

The RDI indicates if one amino acid is emptied faster than the average of amino acids or not. It was previously used by Vachon et al. (1987) to estimate the digestibility of proteins in a digestion cell compared to a reference protein.

RESULTS AND DISCUSSION

Emptying of Whey Proteins and Caseins. After 2 h, the quantities of emptied protidic products corresponded to around 20% of the ingested protein matter for the three diets (Figure 1a). Seven hours after the meal, the protidic matter emptying was slightly higher for Y than for other milks, especially for RSM. However, because of the large variability between experiments and the small number of experiments, differences are not significant. The emptied protidic matter for RSM represented 45% of the ingested amount. Pélissier et al. (1983) found that only 35% of ingested proteic matter was emptied 7 h after the meal in calves with an empty

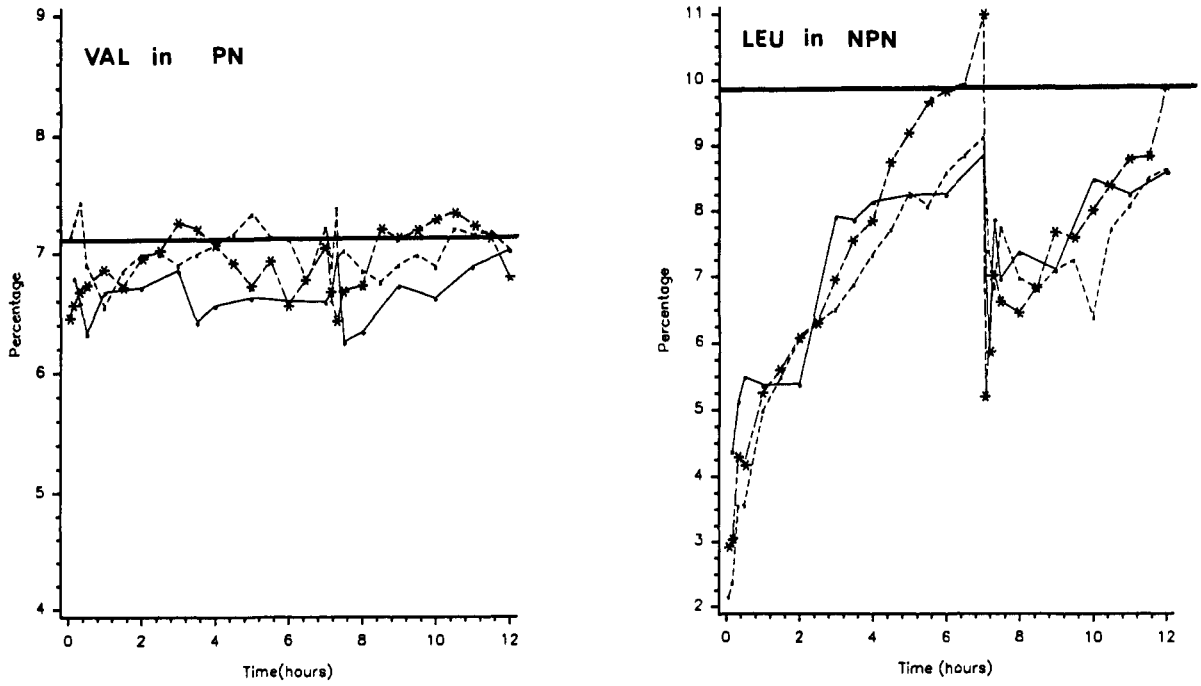

Figure 3. Change with digestion time of amino acid level in digesta (in percentage of the total amino acids). (—) Y; (- - -) RSM; (*) PSM; (—) level in milk.

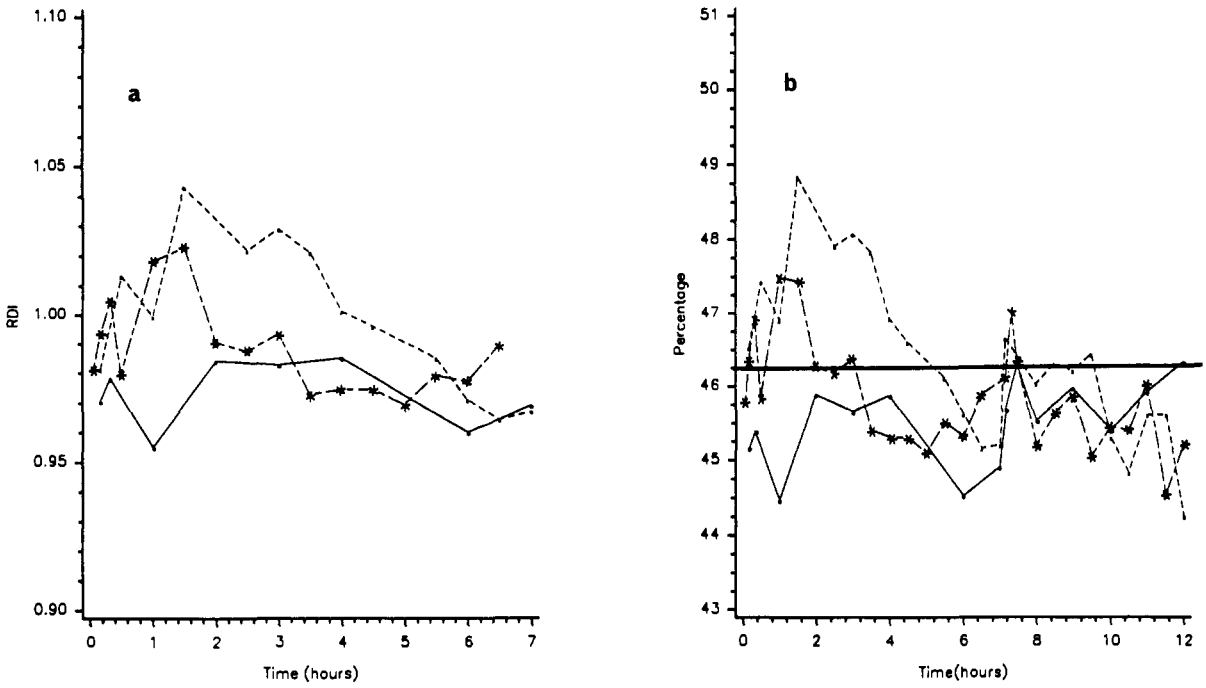

Figure 4. Essential amino acids emptying. (a) Change with digestion time of the RDI for the first meal. (b) Change with digestion time of essential amino acids level (in percentage of total amino acids). (—) Y; (- - -) RSM; (*) PSM; (—) level in milk.

stomach fed milk. Our value was higher, but it was a mean of values having an important variability. For one experiment, we also found 32%.

For Y and PSM the proportion of whey proteins in gastric effluents was the same as in the diet, while with RSM this proportion increased from 15% to 25% (Figure 1b).

The 12% TCA soluble casein in the emptied casein fraction represented 7% 2 h after the meal for all tested diets (Figure 1c). Seven hours after the meal, this proportion was around 20% for Y and PSM, whereas for RSM it was slightly higher.

For Y, casein and whey proteins were evacuated slightly more slowly than the fresh matter, whey emptying being a little faster than casein emptying (Figure 1d). Whey proteins from RSM were also evacuated slightly more slowly than the fresh matter, but casein emptying was slowed down greatly. For PSM, whey proteins as casein were strongly retained in the stomach and less than 50% were emptied after 12 h. The same pattern was also observed 7 h after the first meal.

All of these data are consistent with previous observations (Scanff et al., 1990) and were consequences of the coagulability of the diet. RSM coagulated very quickly in the stomach, and whey proteins were preferentially emptied, casein being emptied later after hydrolysis. PSM coagulated also, but whey proteins were not preferentially emptied because interactions between whey proteins and caseins occurred as a consequence of heating. Heating at 95 °C for 0.5 or 1 min resulted in the association of 58%

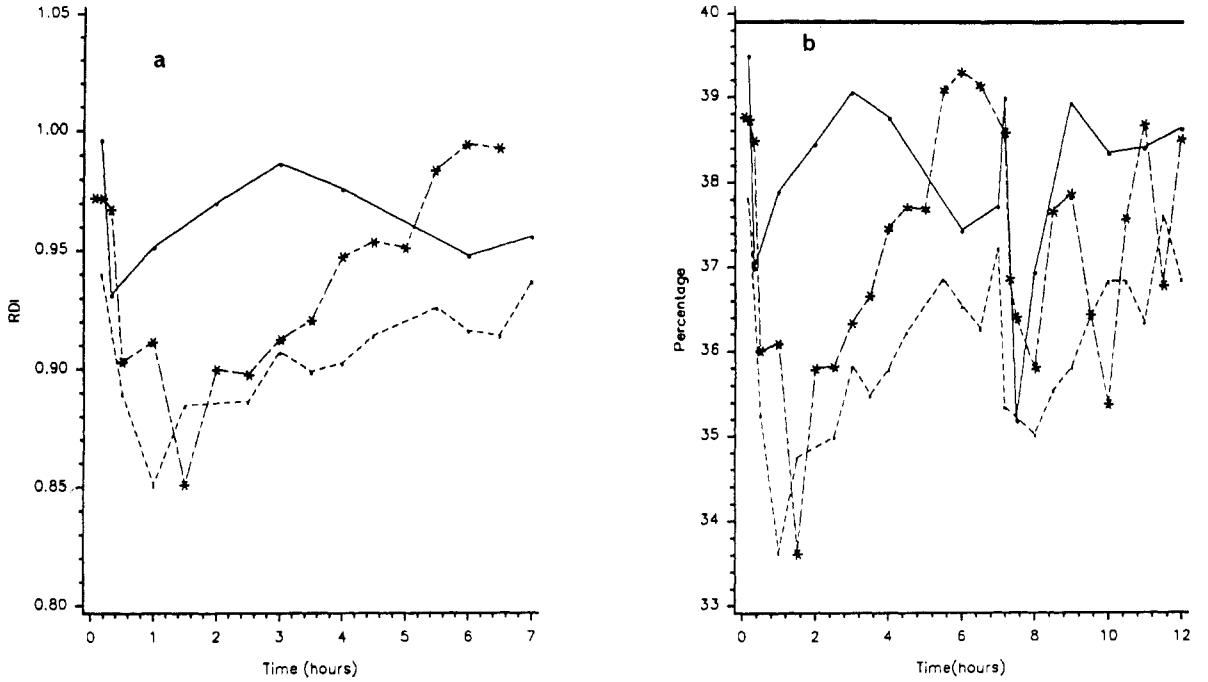

Figure 5. Hydrophobic amino acids emptying. (a) Change with digestion time of the RDI for the first meal. (b) Change with digestion time of hydrophobic amino acids level (in percentage of the total amino acids). (—) Y; (- - -) RSM; (*) PSM; (—) level in milk.

or 66% of the β-lactoglobulin and 8% or 10% of the α-lactalbumin with the casein fraction (Noh and Richardson, 1989). For Y there was no coagulation and all proteins were emptied at the same flow rate with a small speeding up for whey proteins. This is probably due to a separation of the whey and casein fractions in the stomach.

Change with Time of the Amino Acid Composition of Gastric Effluents. The level of each amino acid was determined by acid hydrolysis of the proteins and peptides present in each digesta. The change with time of this level was considered in TCA-soluble (NPN) and in TCA-insoluble (PN) fractions. Different types of behaviors were observed. For several amino acids differences between diets were observed, whereas for others the same pattern was found whatever the diet. Figure 2 shows examples of the first behavior. The level of Asx (Asp + Asn) in the PN was the same during digestion of Y, while it rapidly increased with RSM and PSM until 1.5 h after the meal and slowly decreased to a value close to the level of Asx in the diet. The same observation was made with the second meal. The level of Pro in PN of Y digesta did not change with digestion time, while for the other diets it changed exactly in the opposite way of Asx. These observations were consistent with the coagulability of the diet since the levels of Asx and Pro are very different in whey and casein fractions (less Pro in whey than in casein and more Asx). Consequently, these amino acids could be considered markers of coagulation.

Similarly, the change with time of the level of Thr in NPN or PN was very particular (same pattern for Ile also). Yvon and Pélissier (1987) demonstrated that caseinomacropeptide (CMP) was the first peptide to be released during 2 h after the meal from the stomach of calves fed skim milk. The nonglycosylated fraction of CMP and glycosylated fractions are found in PN and NPN, respectively, and CMP contains a great proportion of Thr (17% in CMP vs 4.6% in milk). The observed change with RSM was in agreement with these results, and Thr level could be considered a good marker of the CMP emptying. Consequently, with PSM, CMP might be also preferentially emptied since the change of Thr was the same as with RSM. For Y Thr level was the same during the whole digestion process. However, this level was higher in NPN than in PN (5.5% vs 4.5%). This could be due to the CMP emptying but with Y more products are emptied quickly after feeding and CMP was immediately diluted in the mixture. With the second meal, the same but less pronounced changes were observed because CMP was emptied and diluted with a lot of products from the first meal.

The changes with time of the level of many amino acids were the same for the three diets in NPN (Asx, Ser, Gly, Ala, Val, Met, Leu, Tyr, Phe, His, Lys, Arg) and in PN (Val, Gly, Met, Tyr, His, Lys, Arg). Figure 3 illustrates two examples of change. For Val in PN, the level remained the same during the entire digestion but for Leu in NPN it increased continuously with digestion time. The same increase was observed for Tyr and Phe and was consistent with gastric proteolysis since chymosin or pepsin hydrolyzes preferentially proteins or peptides after hydrophobic or aromatic residues. The same changes were observed with the second meal.

Emptying of Ketogenic and Branched-Chain Amino Acids Determined after Acid Hydrolysis of Effluents. Because of their importance for metabolism, these amino acids have been considered. These two groups of amino acids were emptied as was the average of all amino acids. No modification of the level of these amino acids occurred in digesta with the three diets. The technological treatment of milk did not modify the pool of these amino acids that enter the gut during digestion.

Emptying of Essential Amino Acids Determined after Acid Hydrolysis of Effluents. Essential amino acids were emptied as was the average of all amino acids since the RDI was always around 1 (Figure 4a). These amino acids were similarly represented in the digesta of the three diets during the whole digestion process with a slight tendency to be more represented for RSM especially just after the first meal (Figure 4b). The observed values for the three diets were near to the proportion of essential amino acids in milk (46.2%) and near to the optimal ratio (essential/total amino acids) of an amino acid mixture giving an optimal weight gain, food intake, and protein efficiency ratio (Ikemoto, 1989). Moreover, the proportion of each essential amino acid in the total of essential amino acids remained the same during the digestion of Y and

was close to the optimal ratio required for good growth (FAO, 1986), except for the sulfur-containing amino acids, while just after milk feeding and during the first hours the proportion of Tyr and Phe was low (around 12% vs 15.7% required). In contrast, the level of Thr was high at the same time. The observed change in the concentration of Tyr, Phe, and Thr among the essential amino acids at the gastric level could be compensated by the pancreatic step since Tyr and Phe that are emptied slower at the gastric level are relased faster by pancreatic proteases and the contrary for Thr (Savoie et al., 1988) according to the pancreatic enzyme specificity.

Emptying of Hydrophobic Amino Acids Determined after Acid Hydrolysis of Effluents. Peptides and protein fragments rich in hydrophobic amino acids (Tyr, Phe, Ile, Leu, Val, Pro) were more retained than the others, especially for RSM and PSM (Figure 5a). The observed level of these amino acids in digesta with RSM and PSM was lower than with Y (Figure 5b). With RSM or PSM the level decreased during the first 3 h, and at 2 h they represented only 34% of the digesta (40% in milk). Thereafter, the level increased slowly until 7 h. Hydrophobic amino acids are known to stimulate the pancreatic protein secretion. This has been demonstrated by Meyer et al. (1976). The physiological consequences of the variation of hydrophobic amino acids with RSM and PSM could be that no stimulation of the pancreatic secretions occurred during the first hours and consequently no degradation of whey proteins, especially IgG and lactoferrin, took place (Reiter, 1978). These proteins could therefore have a biological role. After 3 h when the coagulum was digested, many products appeared in the duodenum and the pancreatic secretions could be thus stimulated since the level of these amino acids increased. With Y, digestion products were always emptied in the duodenum and pancreatic secretions could be kept at a higher level because hydrophobic amino acids were always at the highest level.

CONCLUSION

Technological treatments of milk modified its coagulation in the stomach and consequently the nature of the products leaving the stomach during digestion. The quantities and proportion of whey proteins and caseins varied in digesta with the diet. Consequently, the pool of amino acids that enter the duodenum (as peptides or free amino acids) depends on the digestion time and on the diet, whereas the amino acid composition of the three diets was exactly the same. These observed differences at gastric level could be compensated (as for Tyr, Phe, and Thr) or intensified (as for Pro) by subsequent digestion steps, especially by pancreatic digestion. Savoie et al. (1988) demonstrated that Pro was slowly released at the pancreatic level. Since the Pro level in gastric digesta decreased with milks during the first hours, the available Pro for enterocyte would be significantly reduced during this period.

Since dietary amino acids are differently absorbed and metabolized according to the pool that is presented to the enterocyte, all factors affecting digestion kinetics of a protein such as technological treatments are thus determining factors of amino acid bioavailability.

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