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The heat treatment and the gelation are strong determinants of the kinetics of milk proteins digestion and of the peripheral availability of amino acids

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A B S T R A C T

This study aimed to determine the kinetics of milk protein digestion and amino acid absorption after ingestion of four dairy matrices by six minipigs: unheated or heated skim milk and corresponding rennet gels. Digestive contents and plasma samples were collected over a 7 h-period after meal ingestion. Gelation of milk slowed down the outflow of the meal from the stomach and the subsequent absorption of amino acids, and decreased their bioavailability in peripheral blood. The gelled rennet matrices also led to low levels of milk proteins at the duodenum. Caseins and β-lactoglobulin, respectively, were sensitive and resistant to hydrolysis in the stomach with the unheated matrices, but showed similar digestion with the heated matrices, with a heat-induced susceptibility to hydrolysis for β-lactoglobulin. These results suggest a significant influence of the meal microstructure (resulting from heat treatment) and macrostructure (resulting from gelation process) on the different steps of milk proteins digestion.
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1. Introduction

The nutritional evaluation of a protein source is based on its composition of indispensable amino acids, and its digestibility over the whole digestive tract (Schaafsma, 2000). However, it is now well established that the speed of the digestion is an important dimension in the definition of this nutritional value. Indeed, quickly and slowly-digested proteins differently affect the metabolic use of dietary amino acids for protein metabolism (Breen & Phillips, 2011; Dangin et al., 2003). The concept of 'slow' and 'fast' proteins mainly derives from comparisons between purified caseins (slow proteins) and whey proteins (fast proteins) (Boirie et al., 1997): caseins are able to coagulate in the acidic environment of the stomach and β-lactoglobulin remains in the serum phase of the bolus. Caseins are therefore thought to be retained much longer than whey proteins in the gastric compartment, providing a prolonged plateau of moderate hyperaminoacidemia associated to higher protein deposition than whey proteins in young adults (Lacroix et al., 2006). Conversely, in the elderly people, "fast" proteins, such as whey proteins, are more efficient than casein to promote postprandial protein anabolism (Dangin et al., 2003). However, the intrinsic composition of a protein is not the only determinant of its speed of digestion. The structure of the food, also called "food matrix" and defined as the interactions between food components resulting from technological processes during food manufacturing, also impact on this parameter and the subsequent nutrient bioavailability (Parada & Aguilera, 2007; Turgeon & Rioux, 2011).

In this context, the present study aimed to evidence the possibility of modulating the speed of the digestion of dairy products, without changing their biochemical composition, by applying different technological processes acting on the structure of the meal. Two technological treatments commonly used in the food industry were chosen, a thermal treatment and a gelation process, in order to modify both the micro and the macrostructure of the tested matrices. The effects of these structure modifications on the gastric mean retention time, the residual concentrations of milk proteins

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(specifically caseins and β-lactoglobulin) in the small intestine and the postprandial kinetics of aminoacidemia, were investigated in minipigs. This animal model was chosen because its digestive physiology is close to the human one, at least in the upper part of the gut (Rowan, Moughan, Wilson, Maher, & Tasman-Jones, 1994). The results illustrate the strong effects of the food matrix on the gastric emptying, the hydrolysis of the proteins and the kinetics of amino acid absorption.

2. Materials and methods

2.1. General

All procedures were in accordance with the guidelines formulated by the European Community for the use of experimental animals (L358-86/609/EEC, Council Directive, 1986) and the study was approved by the Local Committee for Ethics in Animal Experimentation (Approval CE15-10; CREEA d'Auvergne, Aubière, France).

2.2. Animals and animal housing

The study involved six 18-months-old female adult mini-pigs (20 ± 1 kg). Three weeks before the experimentation, minipigs were surgically fitted with cannulas as described previously by Bauchart et al. (2007) and with a catheter in the abdominal aorta (7 cm inside the blood vessel). They were housed in subject pens (1 × 1.5 m) in a ventilated room with controlled temperature (21 °C). Between the sampling days, they were fed once daily with 400 g/d of a concentrate feed containing 16% protein, 1% fat, 4% cellulose and 5% ash (Porcyprima; Sanders Centre Auvergne, Aigueperse, France) and had free access to water.

2.3. Test meals

Four dairy matrices having a similar composition but differing by their internal structures were prepared from 145 g of Ultra Low Heat (ULH) skim milk powder (4% of humidity, 34% of protein (*N* × 6.25), 54% of lactose, 8% of ash) manufactured at INRA-STLO (Rennes), rehydrated to 1 kg with Milli-Q pure water. A concentration of about 50 g proteins/kg was chosen for the matrices preparation in order to satisfy animal daily protein requirements with 1 kg (2,5 g proteins/kg BW) and to produce dairy gels with sufficient firmness under coagulation conditions (24 h at 20 °C). The coagulation was performed during 24 h because of experimental constraints: meal ingestion occurring at 8:00 a.m., the gelled matrices were prepared the day before the sampling day. The coagulation was also performed at room temperature, in a room located near the housing of mini-pigs. The rehydrated solution was used as liquid matrices, either unheated (**LR**) or heated 10 min at 90 °C (**LH**). The gel matrices were then prepared from LR (rennet DSM Maxiren 180: 0.003 v/w%) (**GR**) or LH (rennet DSM Maxiren 180: 0.3 v/w%) (**GH**). The rennet gelation was performed at 20 °C and launched 24 h before the beginning of the *in vivo* experiment. The four matrices were added with chromium-EDTA, a non-hydrolysable and non-absorbable marker of the liquid phase (110.8 ppm, w/w).

2.4. Experimental procedures

The experimental protocol included two periods: the first one for effluent sampling, the second one for blood sampling. In each one, the four dairy matrices were randomly tested on each minipig. Within a period (2 wk), the days of sampling were separated by at least 2 d. Test meals (1 kg of freshly prepared matrix) were offered for 10 min, and were always consumed entirely within that time. Minipigs had no access to water from 1 h before to 7 h after the meal delivery. Duodenum and jejunum effluent samplings were performed on the same sampling day. Effluents were collected in plastic bottles 30 min before and 0, 20, 50 min, 1 h 45, 2 h 45, 3 h 45, 5 h 15 and 6 h 45min after the beginning of meal ingestion for the duodenum and 15 min before and 15, 35 min, 1 h 05, 1 h 30, 2 h 30, 3 h 30, 5 h and 6 h 30min after the beginning of meal ingestion for the jejunum. The sampling was stopped when 40 ml were collected or after a maximum of 10 min sampling. Collected effluents were weighed, added with a protease inhibitor (0.37 mg phenylmethanesulfonyl fluoride/ml) and homogenised with a stirrer. A first fraction of the homogenate (about 5 g) was used for dry matter determination (24 h at 100 °C). A second fraction (about 20 g) was directly frozen in liquid nitrogen and used for SDS–PAGE and ELISA analyses. The remaining of the homogenate was freeze-dried and used for chromium analysis. Blood sampling was performed 60 min, 30 min before and 15, 30 min, 1, 2, 3, 4 h, 5 h 30min, 7 h after the beginning of meal ingestion. Blood samples (2.7 ml) were collected in tubes containing lithium-heparin (S-monovettes, Sarstedt, Marnay, France) and immediately centrifuged at 3000*g* for 10 min at 4 °C. Supernatant was frozen in liquid nitrogen and stored at −80 °C.

2.5. Sample analysis

2.5.1. Chemical analyses

The total nitrogen (total *N*), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) of the matrices before ingestion were analyzed according to the Kjeldahl method (FIL-IDF international standard 20B:1993 and 135A:1998 and FIL-20-1 ISO8968-1 for total *N*; FIL-IDF international standard 29:1964 and ISO 17997-1 IDF 29-1 for NCN; FIL-IDF international standard 20B:1993 and ISO 8968-4 FIL20-4 for NPN). Freeze-dried duodenal effluents were used for chromium analysis. After homogenisation, 1 g of effluent was ashed at 550 °C for 12 h. Chromium was extracted from ashes using 10 ml of an acidic mixture (HCl 1.5 mol/l, HNO_3 1.5 mol/l). The mixture was filtered, added with 5 ml of KCl 0.5 mol/l, and diluted to 50 ml with distilled water. The chromium content was then determined using an atomic absorption spectrometer (AAnalyst 400 Spectrometer, PerkinElmer, Courtaboeuf, France). For amino acid analyses, after thawing, 500 μl of plasma were deproteinised with dried sulphosalicylic acid (55 mg/ml plasma), after the addition of norleucine (50 μl, 1.25 mmol/l), used as an internal standard. Samples were mixed and allowed to stand 15 min at room temperature. Precipitated proteins were removed by centrifugation (10,000*g* for 10 min at 4 °C). The supernatant was then diluted (2/3) with the lithium injection buffer (Bioritech, Guyancourt, France), containing glucosaminic acid, used as an injection standard. Plasma amino acid concentrations were determined with an amino acid analyzer (Bio-Tek Instruments A.R.L., St. Quentin Yvelines, France) by ion-exchange chromatography, with post-column derivatisation with ninhydrine (Slocum & Cummings, 1991). For LR and GR matrices, cholecystokinin (CCK) and ghrelin concentrations in plasma were measured with ELISA kits (BlueGene Biotech CO., Shanghai).

2.5.2. Rheological measurement of the formation of rennet milk gels

Formation of the rennet-induced gels was monitored by measuring the elastic modulus (G′), the viscous modulus (G″) and the loss tangent (tan δ, defined as the ratio of the viscous to the elastic modulus of a system, G″/G′) according to the procedure previously described by Morand, Guyomarc'h, Pézennec, and Famelart (2011) at 20 °C with a strain of 0.1%. The onset of gelation was defined as the time when G′ > 1 Pa. After 24 h of gelation, the effect of the time scale of the applied strain on the rheological properties of

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