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Acid and rennet gels exhibit strong differences in the kinetics of milk protein digestion and amino acid bioavailability

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ABSTRACT

This study aimed at determining the kinetics of milk protein digestion and amino acid absorption after ingestion by six multi-canulated mini-pigs of two gelled dairy matrices having the same composition, similar rheological and structural properties, but differing by their mode of coagulation (acidification/renneting). Duodenal, mid-jejunal effluents and plasma samples were collected at different times during 7 h after meal ingestion. Ingestion of the acid gel induced a peak of caseins and β-lactoglobulin in duodenal effluents after 20 min of digestion and a peak of amino acids in the plasma after 60 min. The rennet gel induced lower levels of both proteins in the duodenum (with no defined peak) as well as much lower levels of amino acids in the plasma than the acid gel. Plasma ghrelin concentrations suggested a potentially more satiating effect of the rennet gel compared to the acid gel. This study clearly evidences that the gelation process can significantly impact on the nutritive value of dairy products.

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1. Introduction

Food proteins provide amino acids for protein synthesis and other nitrogen-containing compounds. They undergo digestion in the gastrointestinal tract, which involves different mechanical and enzymatic processes like grinding in the mouth, mixing in the stomach, gastric emptying, hydrolysis achieved by gastric and pancreatic enzymes, transit and absorption in the small intestine (Kong & Singh, 2008). The nutritional properties of food have been largely assessed according to their composition in macronutrients i.e., proteins, lipids, carbohydrates, etc. However, recent works have demonstrated that micronutrients bioaccessibility and bioavailability is also strongly influenced by the food microstructure (Parada & Aguilera, 2007) although the effect of the food matrix structure on the different steps of protein digestion has rarely been addressed. The impact on nutrients bioaccessibility of food matrix, resulting from technological processes and from physiological parameters like oral mastication (Rémond et al., 2007), has mainly been investigated *in vitro* for food such as carrot (Kong & Singh, 2011), almond (Kong & Singh, 2009), meat (Remond, Savary-Auzeloux, Gatellier, & Sante-Lhoutellier, 2008) and spinach (Castenmiller, West, Linssen, van het Hof, & Voragen, 1999).

Among food proteins, bovine milk proteins constitute a high-grade source of dietary nitrogen for humans and are of major interest due to the worldwide consumption of various dairy processed products (fermented milk, cheese, yogurt, etc.). In addition to their nutritional function, milk proteins are precursors of peptides exhibiting bioactive signals involved in different physiological functions (immunomodulatory, opioïd, anxiolytic, antihypertensive, antimicrobial, etc.) (Korhonen, 2009). The main milk proteins are caseins (80%) and whey proteins (20%), of which β-lactoglobulin is the major one (Farrell et al., 2004). Caseins have a highly flexible structure that makes them sensitive to proteolysis. They are associated into supramolecular complexes, called casein micelles.

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Conversely, β-lactoglobulin has a definite globular structure that makes it more resistant to proteolysis (Kim et al., 2007).

Interestingly, caseins and β-lactoglobulin are known as "slow" and "fast" proteins, respectively, due to their different behaviours related to gastric emptying (Boirie et al., 1997). Casein micelles form a coagulum in the acidic environment of the stomach whereas β-lactoglobulin remains in the serum phase of the bolus that is rapidly evacuated from the stomach to the small intestine. Caseins are therefore thought to be retained much longer than whey proteins in the gastric compartment.

Leucine is an amino acid able to stimulate muscle protein synthesis. Several studies have indeed demonstrated that leucine, when administrated as a purified amino-acid, could efficiently restore muscle protein synthesis in elderly people (Rieu et al., 2006). β-lactoglobulin is rich in leucine and has thus been extensively studied in the context of sarcopenia, a pathological state affecting elderly people (Rieu et al., 2007). In the daily life however, leucine is consumed by humans via proteins that are included in a food matrix and the impact of the food structure on the ability of leucine to restore protein synthesis has never been investigated so far.

Recently, we have clearly demonstrated that the heat-treatment of milk and rennet coagulation had a significant impact on the bioavailability of dietary amino acids (Barbé et al., 2013). While milk goes quickly through the stomach resulting in a rapid increase in amino acid concentration in the plasma, rennet gel is retained longer in the stomach delaying the release of amino acids in the plasma. In this study raw and heated milks were compared to their rennet gel counterparts but, to our knowledge, the influence of the coagulation mode of milk on amino acids bioavailability has never been assessed so far.

The aim of the present study is therefore to compare the influence of two gelled dairy matrices, having the same composition, similar rheological properties but differing by their mode of coagulation, on milk protein digestion and amino acid absorption. Two different gel structures were thus designed: an acid gel (AG) and a rennet gel (RG). The gastric mean retention time of each matrix was estimated from the concentrations measured at the stomach exit of a non-hydrolysable and non-absorbable marker of the meal. Amino acid concentrations in the plasma as well as residual concentrations of intact proteins (caseins and β-lactoglobulin) at both the duodenum and the mid-jejunum were measured over a 7 h postprandial period. Mini-pigs were used for this study as they are thought to be good models of the human digestive physiology (Rowan, Moughan, Wilson, Maher, & Tasmanjones, 1994). A potential contribution of the meal to satiety was also determined by measuring the two gastrointestinal hormones, CCK (cholecystokinin) and ghrelin, in the plasma.

2. Materials and methods

2.1. Animals and animal housing

All procedures were in accordance with the guidelines formulated by the European Community for the use of experimental animals (EU Directive 2010/63/EU) and the study was approved by the Local Committee for Ethics in Animal Experimentation (Approval CE15-10; CREEA d'Auvergne, Aubière, France). The detailed protocol concerning the *in vivo* experiments and the analytical procedures used to characterise the recovered samples were previously published (Barbé et al., 2013). Only a brief summary is therefore reported here.

The study involved six 18-months-old female adult mini-pigs (20 ± 1 kg) surgically fitted with one duodenal and one jejunal cannulas as described previously (Bauchart et al., 2007) and with a catheter in the abdominal aorta.

2.2. Test meals

Two dairy gels having a similar composition but differing by their mode of coagulation were prepared in our pilot plant facilities. 145 g of Ultra Low Heat skim milk powder (4% of humidity, 34% of protein (N $\times$ 6.25), 54% of lactose, 8% of ash) manufactured at INRA-STLO (Rennes) were rehydrated to 1 kg with Milli-Q pure water. A meal mass of 1 kg with a concentration of about 50 g proteins/kg was chosen for the dairy gels in order to satisfy animal daily protein requirements (2.5 g proteins/kg body weight) and to produce gels with sufficient firmness under coagulation conditions (24 h at 20 °C). The rehydrated solution was heat-treated 10 min at 90 °C. The gel matrices were then prepared from this solution, either by acid coagulation (AG, glucono-δ-lactone (GDL): 3% w/w) or by rennet coagulation (RG, rennet DSM Maxiren 180: 0.3% v/w). The two matrices were added with chromium-EDTA, a non-hydrolysable and non-absorbable marker of the liquid phase (110.8 ppm, w/w).

2.3. Experimental procedures

The experimental protocol included 2 periods: the first one for effluent sampling, the second one for blood sampling. In each one, the two dairy matrices were randomly tested on each minipig. Within a 2 week-period, the days of sampling were separated by at least 2 d. Test meals (1 kg of freshly prepared AG or RG) 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 as described previously (Barbé et al., 2013). 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. Supernatants were frozen in liquid nitrogen and stored at −80 °C.

2.4. Sample analysis

2.4.1. Chemical analyses

Chromium and amino acids were quantified in effluents and plasma respectively as described previously (Barbé et al., 2013). Cholecystokinin (CCK) and ghrelin concentrations in plasma were measured with ELISA kits (BlueGene Biotech CO., Shanghai). Casein and β-lactoglobulin were determined on duodenal and jejunal samples by inhibition ELISA as previously described (Dupont et al., 2010).

2.4.2. Rheological measurements

Formation of the 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 (Morand, Guyomarc'h, Pezennec, & Famelart, 2011) at 20 °C with a strain of 0.1%. Rheological properties of the gels were monitored by compression test as described previously (Barbé et al., 2013).

2.4.3. Image analysis of the milk gels microstructure

Gel microstructure was visualised by confocal microscopy as described previously Morand, Guyomarc'h, Legland, and Famelart (2012). Grey level granulometric methods from mathematical morphology were applied to determine the characteristic pore size of both gels as described (Devaux, Bouchet, Legland, Guillon, & Lahaye, 2008) and explained (Morand, Guyomarc'h, Legland, & Famelart, 2012) previously.

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