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## **In vitro infant digestion model leads to similar conclusion as in vivo study: focus on human milk and infant formula protein digestion**

E Charton, Olivia Ménard, Marie-Françoise Cochet, Yann Le Gouar, Julien Jardin, Gwénaële Henry, Jordane Ossemond, Paul J. Moughan, Carlos Montoya, Amandine Bellanger, et al.

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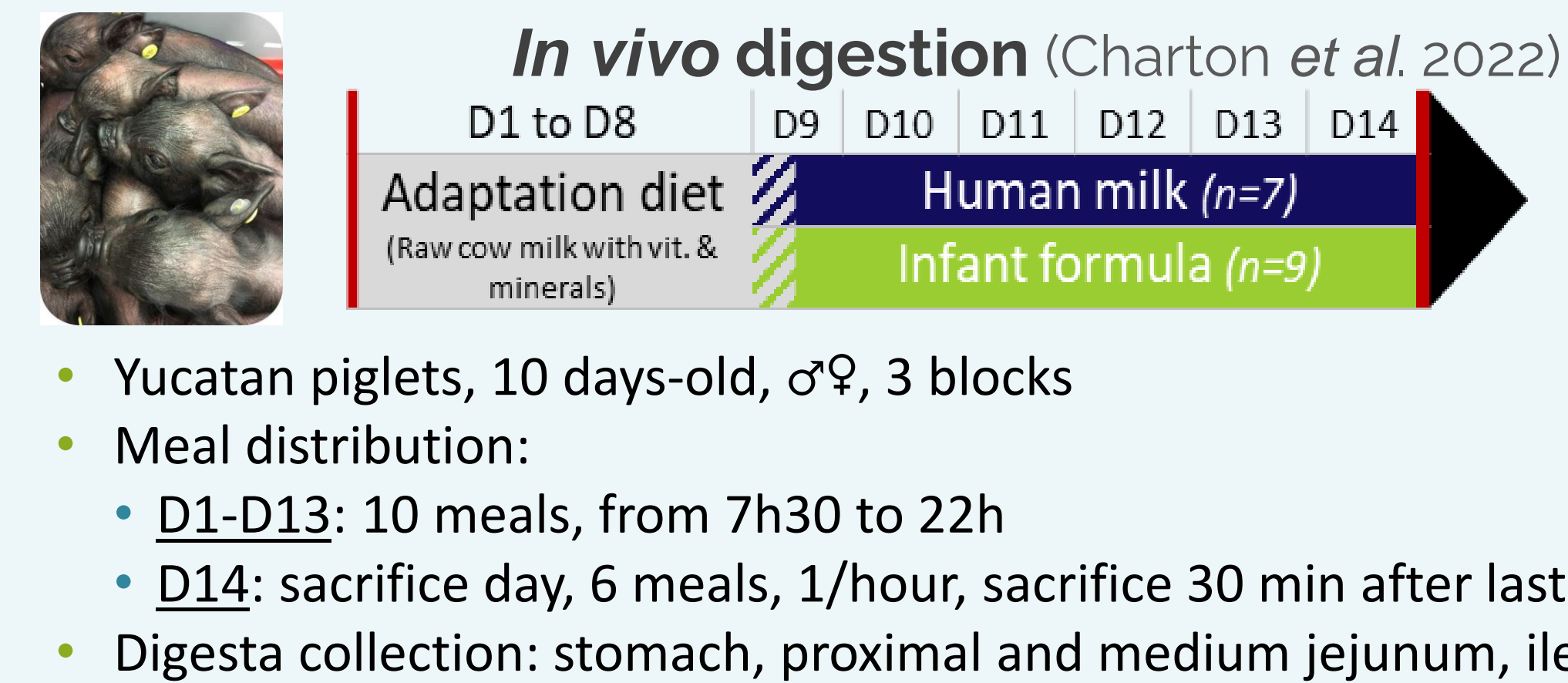
## INTRODUCTION AND OBJECTIVES

- ✓ Infant formula (IF) is the adequate Human milk (HM) substitute despite of still remaining differences in fine composition and structure.
- ✓ HM and IF are assumed to have different digestion kinetics although they are rarely directly compared either *in vivo* or *in vitro*.

→ The present study aimed to evaluate the digestion kinetics and the structure evolution using the DIDGI® dynamic digestion system at the infant stage.

## MATERIALS AND METHODS

- Human Milk:**  
Pool of 50 raw milk samples  
Lactation time: 1.8 - 2 months post-delivery  
**1.0% true proteins, 2.8% lipids**
- Infant formula:**  
NativIF basic IF powder (Yu *et al.*, 2021)  
Rehydrated at **1.4% true proteins, 3.2% lipids**



DIDGI® system

## In vitro digestion

- Parameters based on literature** (Roman *et al.*, 2007 ; Bourlieu *et al.*, 2014):
- Gradual decrease of gastric pH  
→  $pH = 8 \times 10^{-5} \times time^2 - 0,031 \times time + pH_{meal}$
  - Enzymes: Rabbit Gastric Extract + Porcine pancreatin. Bovine bile
  - Gastric emptying by Elashoff fitting (half-time emptying –  $T_{1/2, HM} = 47$  min ;  $T_{1/2, IF} = 78$  min).
  - Sampling time: diet (G0), gastric phase (G20, G40, G80, G120, G180\*), intestinal phase (I20, I40, I80, I120, I180) \*only for IF sampling

## MACROSCOPY Scale

### Evolution of the matrix structure

Laser light scattering, Confocal microscopy (Confocal Zeiss)

## MOLECULAR scale

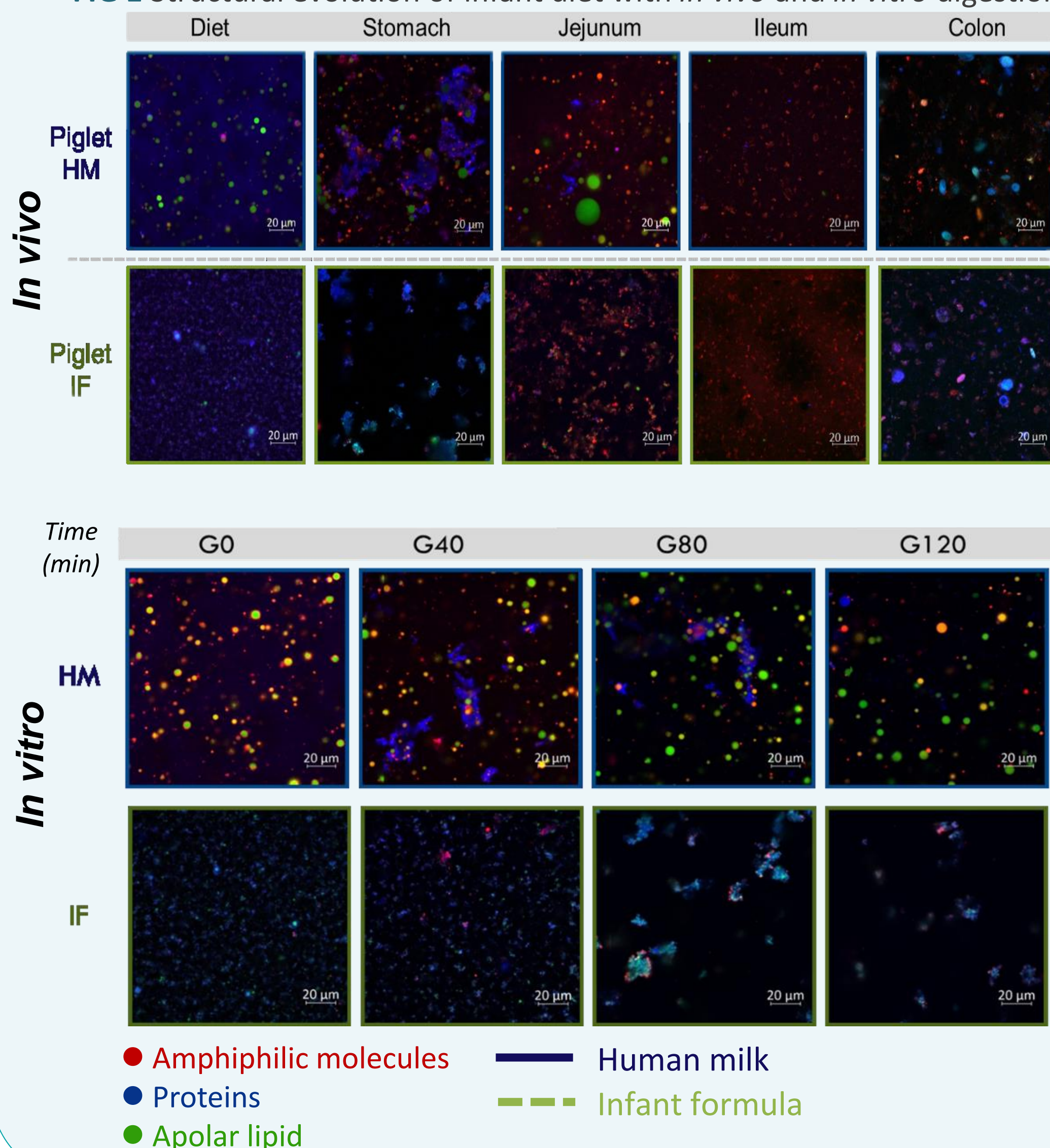
### Proteolysis

Gas chromatography, SDS-Page, OPA, Amino acid bioavailability

## RESULTS

### STRUCTURE

**FIG 1** Structural evolution of infant diet with *in vivo* and *in vitro* digestion.



**Table 1** Particle size characteristics (mode, in  $\mu\text{m}$ ) of samples during *in vivo* and *in vitro* gastric digestion of infant diet.

	In vivo		In vitro				
	Undigested diets	Stomach	G0	G40	G80	G120	
HM	4.74 ± 0.21	95.40 ± 15.01	5.14 ± 0.08	25.18 ± 9.38	23.34 ± 7.69	21.95 ± 7.34	
IF	0.58 ± 0.005	45.50 ± 0.93	0.16 ± 0.002	5.87 ± 0.76	22.21 ± 3.57	24.71 ± 3.67	
p-value	<0.001	0.009	<0.001	0.007	0.760	0.457	

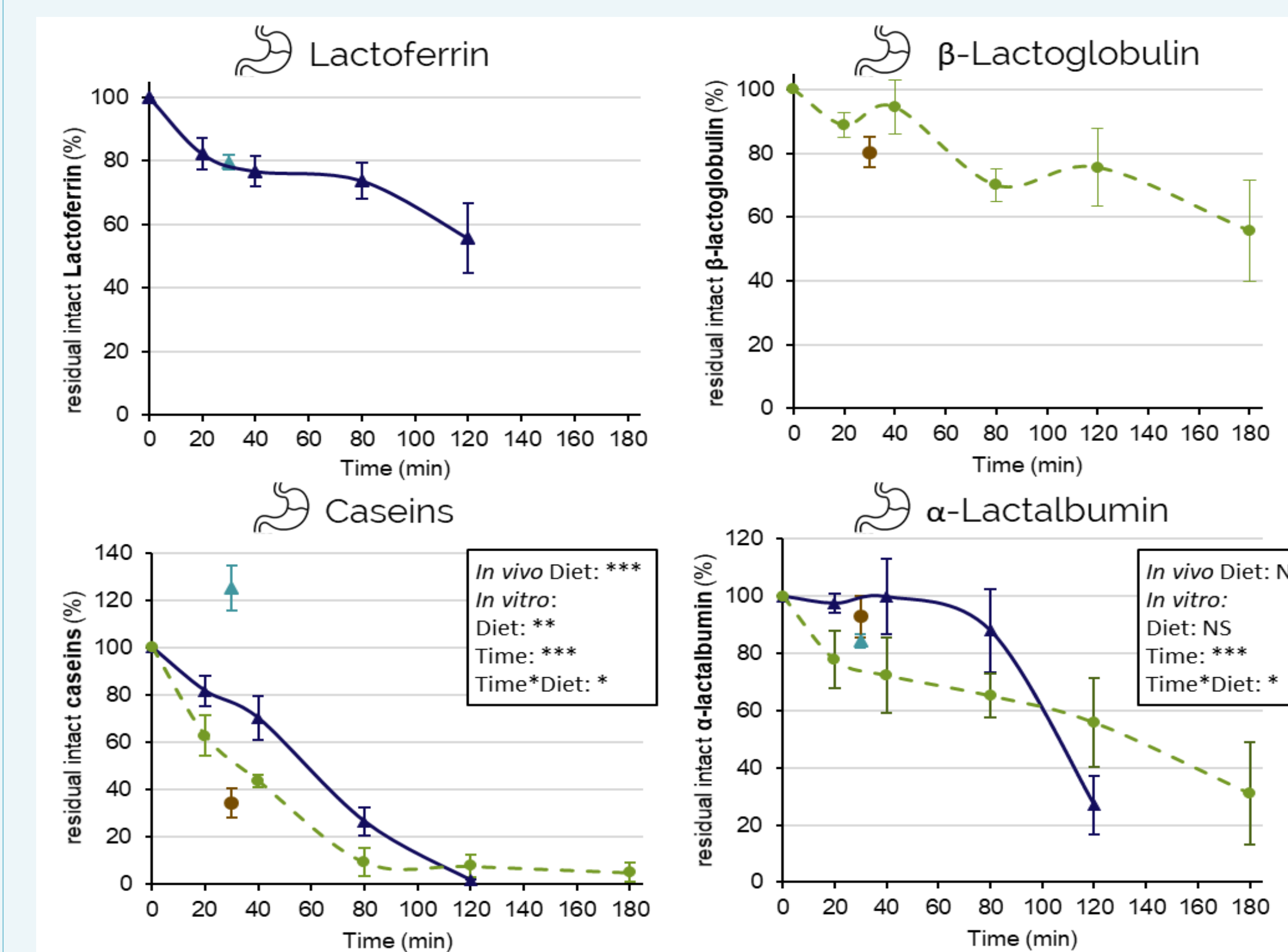
### Structure highlights :

- HM fat globules were sized around 5  $\mu\text{m}$  while IF fat droplets were sized under 1  $\mu\text{m}$ . HM fat globules remained present across *in vivo* or *in vitro* digestion.
- Particle aggregation, specifically protein ones, was faster in stomach during *in vitro* HM digestion (40 min) than in IF digestion (80 min).
- *In vivo* stomach phase structure (30 min) corresponded to *in vitro* gastric phase between G40 and G80 → role of enzyme and pH.
- Final aggregate sizes were more heterogenous for HM especially due to protein aggregation and at a lesser extent to native fat globules in HM.

## PROTEOLYSIS

Representation corrected by meal dilution and emptying (Mean ± SEM; NS, non significant; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001)

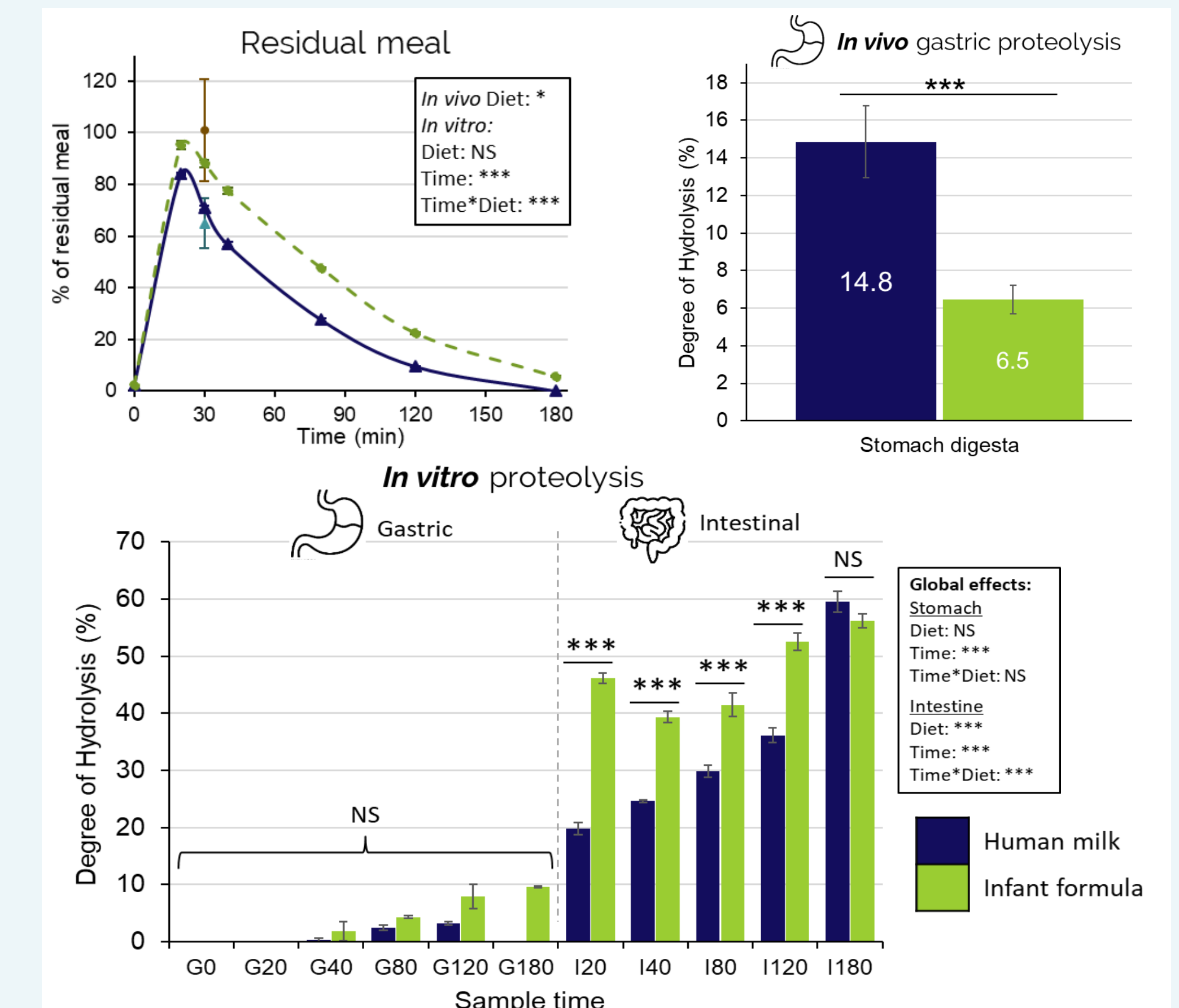
**FIG 2** *In vivo* and *in vitro* gastric residual intact protein.



### Proteolysis highlights:

- No significant difference for gastric residual studied protein except caseins between diets and digestion model for each infant diet.
- Higher concentration of caseins in *in vivo* chyme was related to different clot structures and repeated feeding compared to *in vitro* digestion

**FIG 3** *In vivo* and *in vitro* gastric residual meal and proteolysis degree



- Proteolysis was significantly higher in HM than IF during *in vivo* gastric digestion while no difference was found *in vitro*.
- Intestinal proteolysis was lower in HM at I20, I40 and I120 indicating a faster proteolysis for IF during the first digestion times.

## SUMMARY

- ✓ Digesta structure of HM and IF differed in the upper part of the digestive tract but not in distal intestine.
- ✓ Protein digestion kinetics determined *in vitro* slightly differed to that determined *in vivo*.
- ✓ Protein digestion depended on protein nature.
- ✓ Present *in vitro* digestion model is a good tool to better understand *in vivo* digestion of HM and IF, especially regarding gastric digestion.

## CONCLUSIONS

Despite nutritional similarity, this study highlights the influence of the matrix on the structure of the digesta and on the digestion kinetics. The present *in vitro* digestion model is a good tool to better understand *in vivo* digestion.