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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

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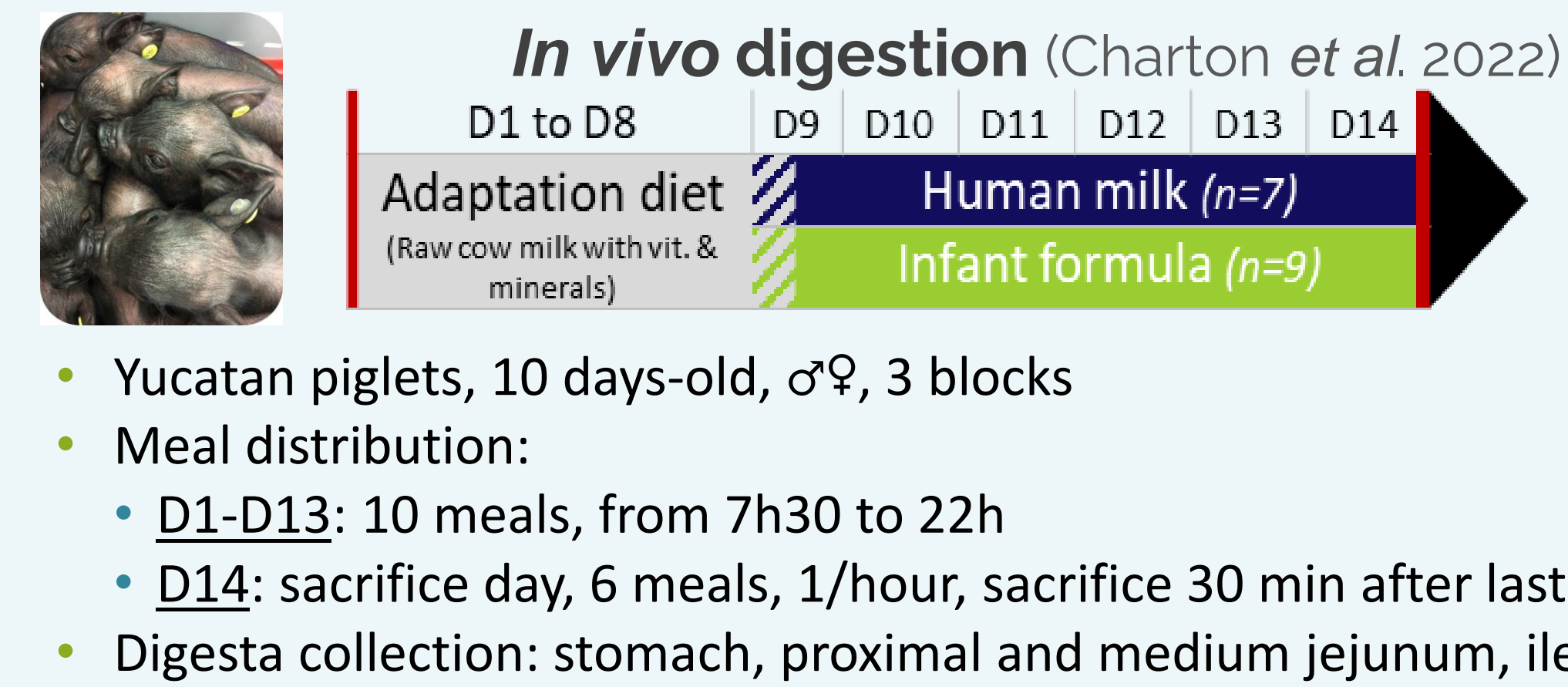
Abstract #: 1380T

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.

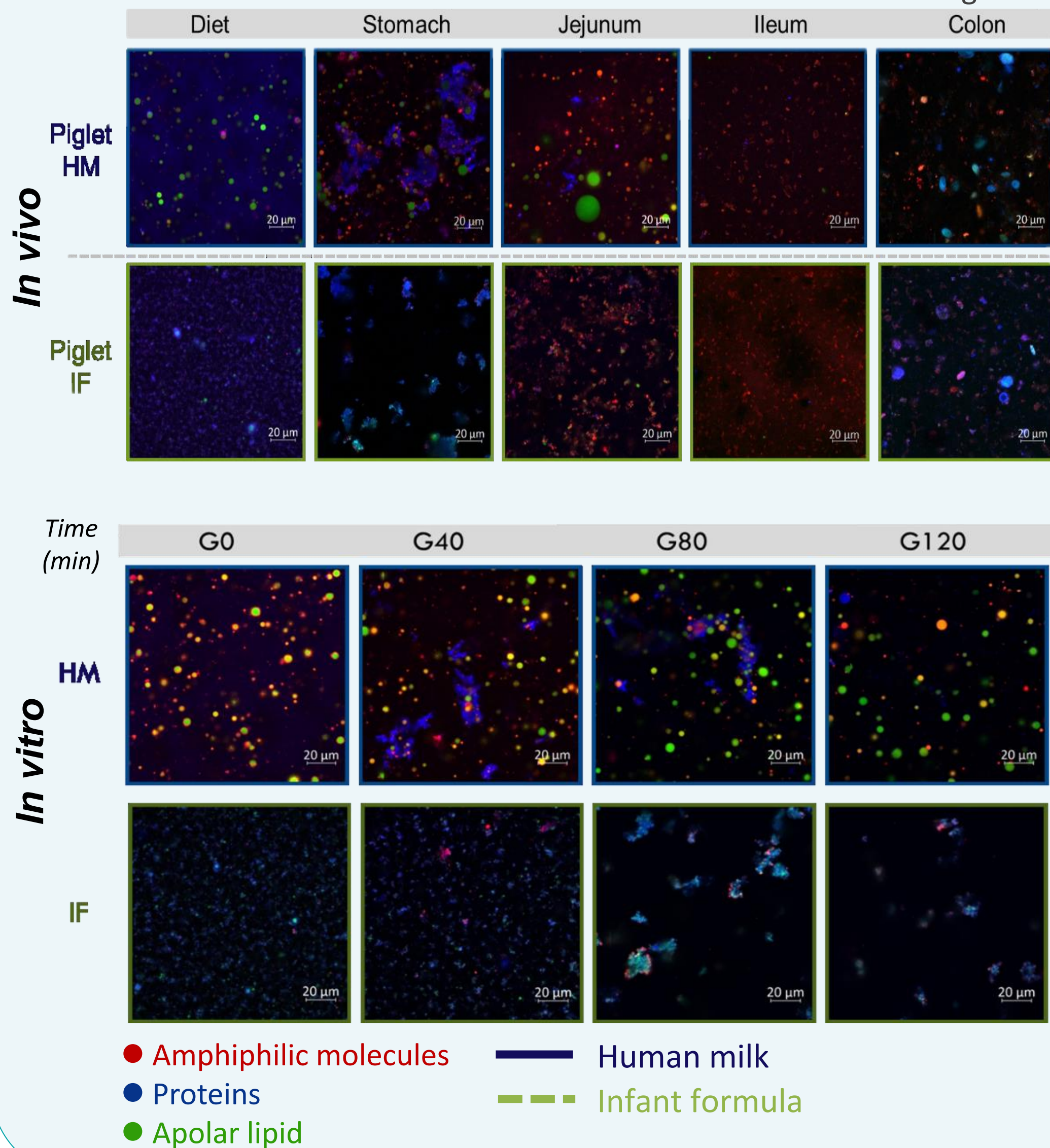


Table 1 Particle size characteristics (mode, in μm) of samples during *in vivo* and *in vitro* gastric digestion of infant diet.

	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	

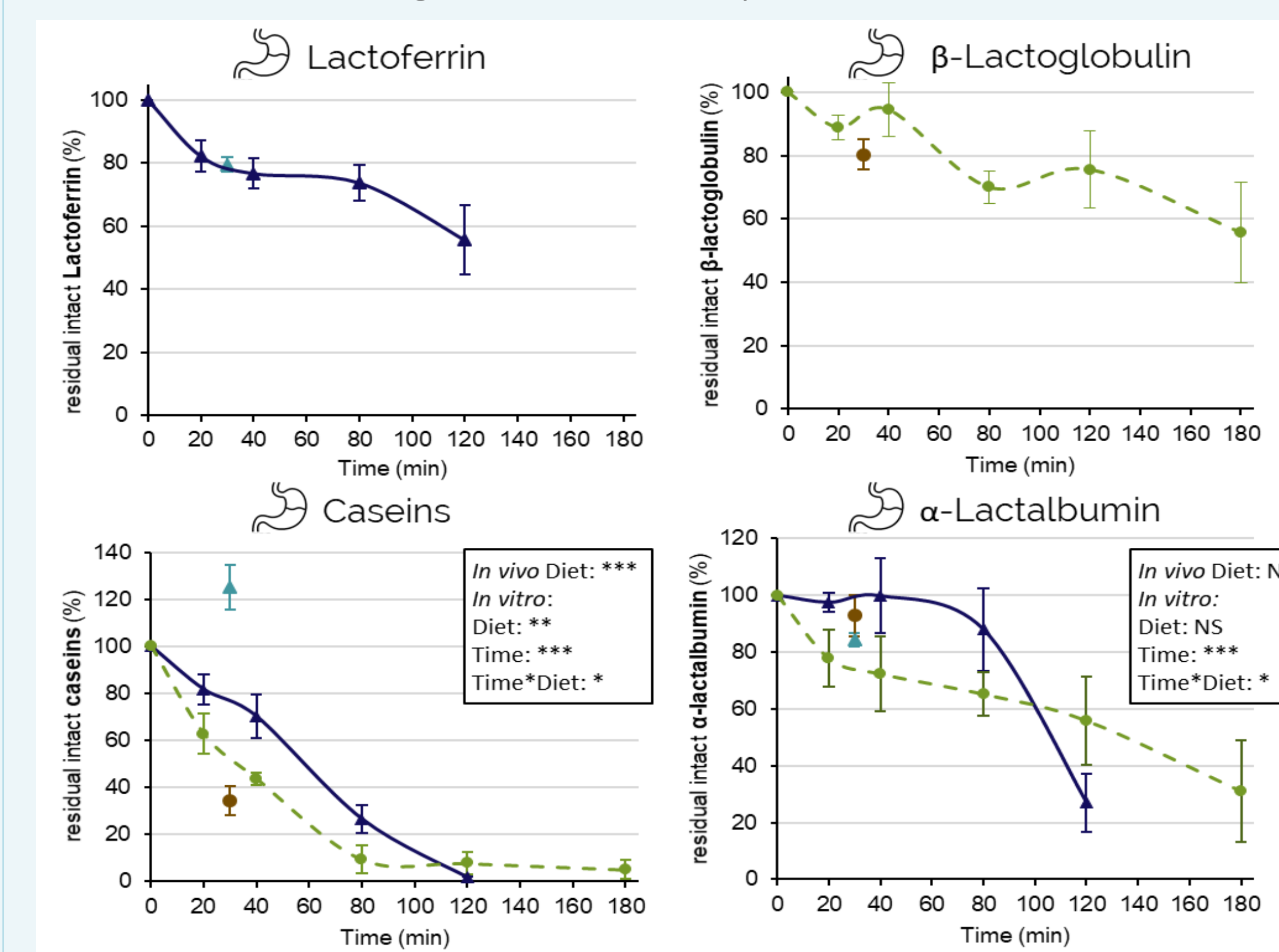
Structure highlights :

- HM fat globules were sized around 5 μm while IF fat droplets were sized under 1 μm . 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 \pm SEM; NS, non significant; *, P<0.05; **, P<0.01; ***, P<0.001)

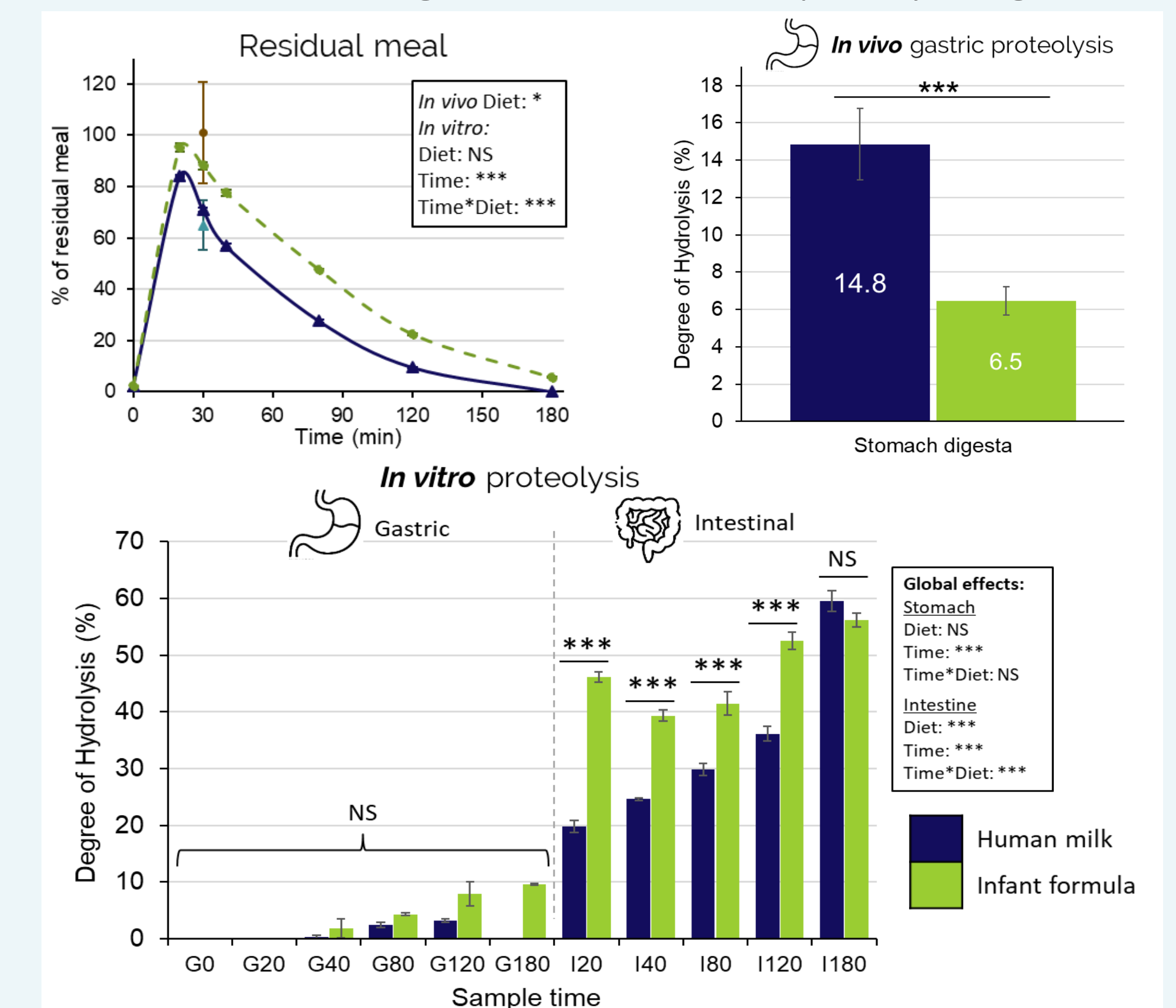
FIG 2 *In vivo* and *in vitro* gastric residual intact protein.



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.