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**

In vivo digestion (Charton *et al.* 2022)

D1 to D8	D9	D10	D11	D12	D13	D14
Adaptation diet (Raw cow milk with vit. & minerals)	Human milk *(n=7)*					
	Infant formula *(n=9)*					

- Yucatan piglets, 10 days-old, ♂♀, 3 blocks
- Meal distribution:
  - D1-D13: 10 meals, from 7h30 to 22h
  - D14: sacrifice day, 6 meals, 1/hour, sacrifice 30 min after last meal,
- Digesta collection: stomach, proximal and medium jejunum, ileum

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 → **MOLECULAR scale**

Evolution of the matrix structure
Laser light scattering, Confocal microscopy (Confocal Zeiss)

Proteolysis
Gas chromatography, SDS-Page, OPA, Amino acid bioavalaibility

RESULTS

STRUCTURE

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

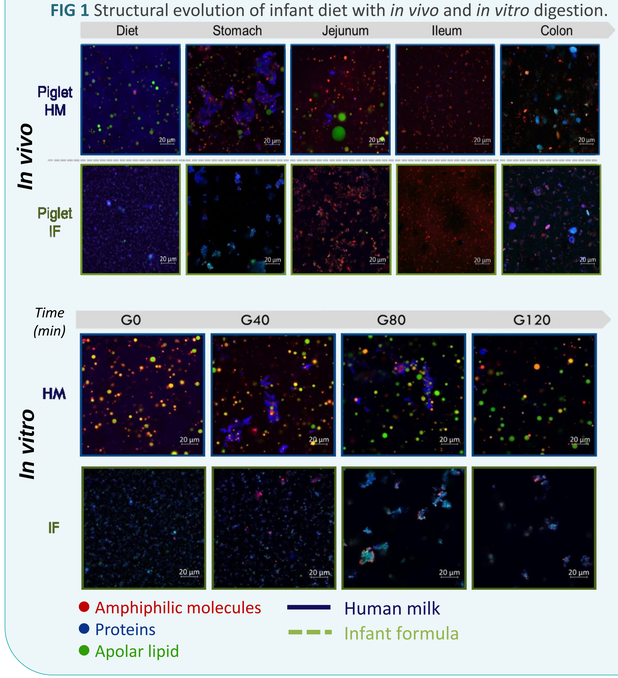
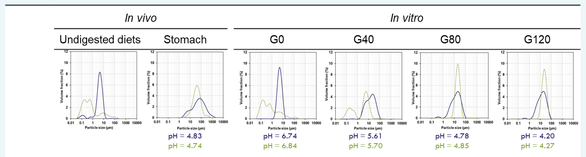


Table 1 Particle size characteristics (mode, in µm) of samples during *in vivo* and *in vitro* gastric digestion of infant diet.



	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

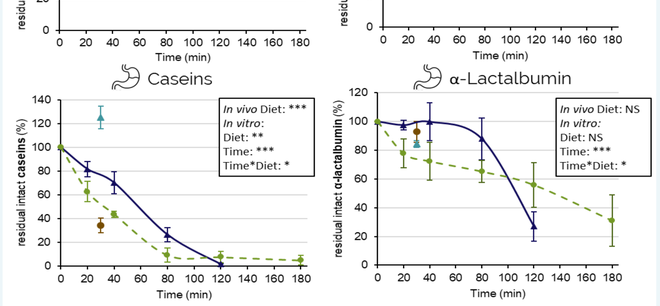
Structure highlights :

- **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 ± SEM;* NS, non significant; *, P<0.05; **, P<0.01; ***, P<0.001)

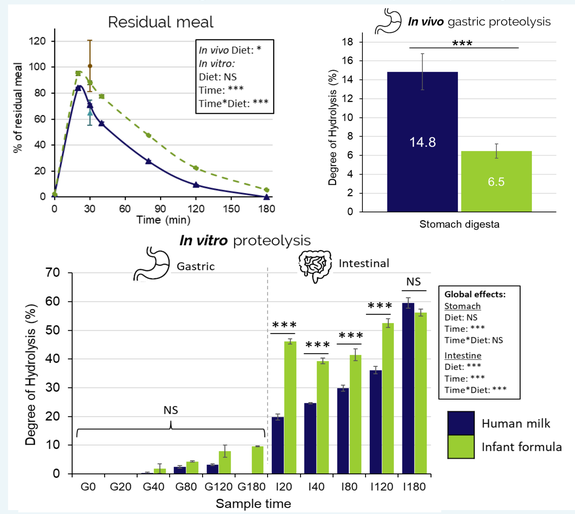
FIG 2 *In vivo and in vitro* gastric residual intact protein.



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.

Bourlieu C, Ménard O, Bouzerzour K, *et al*. Specificity of Infant Digestive Conditions: Some Clues for Developing Relevant In Vitro Models. *Crit Rev Food Sci Nutr* (2014).
Charton E, Bourgeois A, Bellanger A, *et al*. Infant nutrition affects the microbiota-gut-brain axis: Comparison of human milk vs. infant formula feeding in the piglet model. *Front Nutr* (2022)
Roman C, Carriere F, Villeneuve P, *et al*. Quantitative and qualitative study of gastric lipolysis in premature infants: do MCT-enriched infant formulas improve fat digestion? *Pediatr Res* (2007).
Yu X, Leconte N, Méjean S, *et al*. Semi-industrial production of a minimally processed infant formula powder using membrane filtration. *J Dairy Sci* (2021).

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