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# Protein ingredient quality within infant formulas impacts digestion and amino acid bioavailability: a combined *in vitro* and *in vivo* approach.

L. Chauvet<sup>1,2,3</sup>, Y. Le Gouar<sup>1</sup>, O. Menard<sup>1</sup>, M.-F. Cochet<sup>1</sup>, A. Brunel<sup>1</sup>, J. Jardin<sup>1</sup>, S. Guérin<sup>2</sup>, R. Janvier<sup>2</sup>, A. Cahu<sup>2</sup>, T. Croguennec<sup>1</sup>, M. Van Audenhaege<sup>3</sup>, D. Dupont<sup>1</sup>, I. Le Huërou-Luron<sup>2</sup>, M. Lemaire<sup>3</sup>, A. Deglaire<sup>1</sup>

<sup>1</sup>INRAE, Institut Agro, STLO, 35042 Rennes, France; <sup>2</sup>Institut NuMeCan, INRAE, INSERM, Univ Rennes, 35590 Saint Gilles, France; <sup>3</sup>Centre Recherche & Innovation, SODIAAL International, 35000 Rennes, France

Abstract #: 1396T

## INTRODUCTION AND OBJECTIVES

- ✓ **Infant formula (IF)** is the only adequate substitute to **Human milk (HM)** even though **differences of fine composition and structure are still remaining**.
  - ✓ **IF** is a **complex matrix** that require **numerous ingredients** and **processing steps**.
  - ✓ **Protein ingredients quality differs** depending on their **origin** (whey vs. ideal whey) and
- The present study aimed to **evaluate how protein ingredient quality (structure and composition) within IF modulates its structure, digestive kinetics and plasma amino acid (AA) content**

## MATERIALS AND METHODS

### SEMI-INDUSTRIAL PRODUCTION OF 4 IFs (A/B/C/D)

- **Ingredients:** **Commercial whey proteins (WPs) ingredients with different origin** (cheese : IFs-A & -B vs. ideal whey IFs-C & -D) and **structure**. **Casein with different supramolecular organization** (micellar : IFs-A, -B & -C vs. non micellar IF-D)
- **Processing:** **Same processing route**, representative of industrial methods



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### IN VITRO DYNAMIC DIGESTION (Chauvet et al., 2023)

#### Model of a 4-week old infant

- Sampling time: diet (G0), gastric phase (G20, G40, G80, G120, G180), intestinal phase (I20, I40, I80, I120, I180)

(Ménard et al., 2015; De Oliveira et al., 2016)

#### MACROSCOPIC MICROSCOPIC SCALE

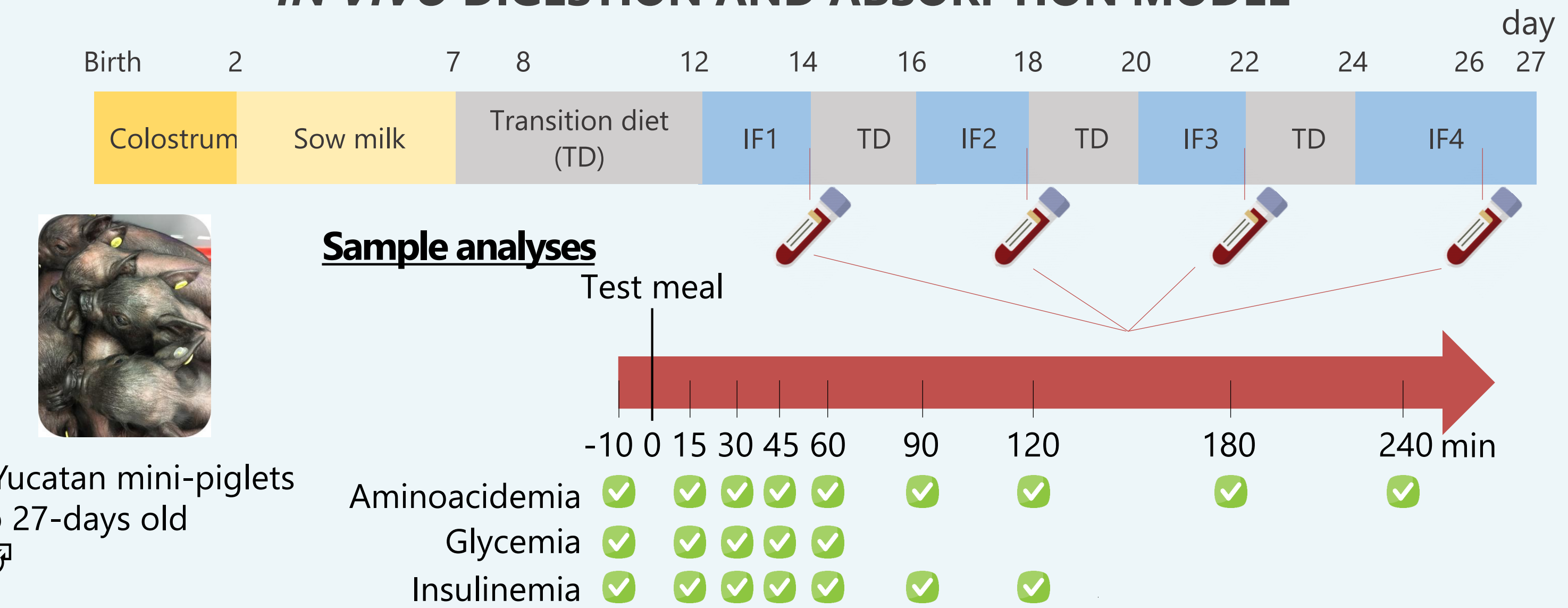
#### Evolution of the matrix structure

- Laser light scattering
- Confocal microscopy

#### Proteolysis

- SDS-PAGE
- LC-MS/MS
- OPA
- Ion-exchange chromatography

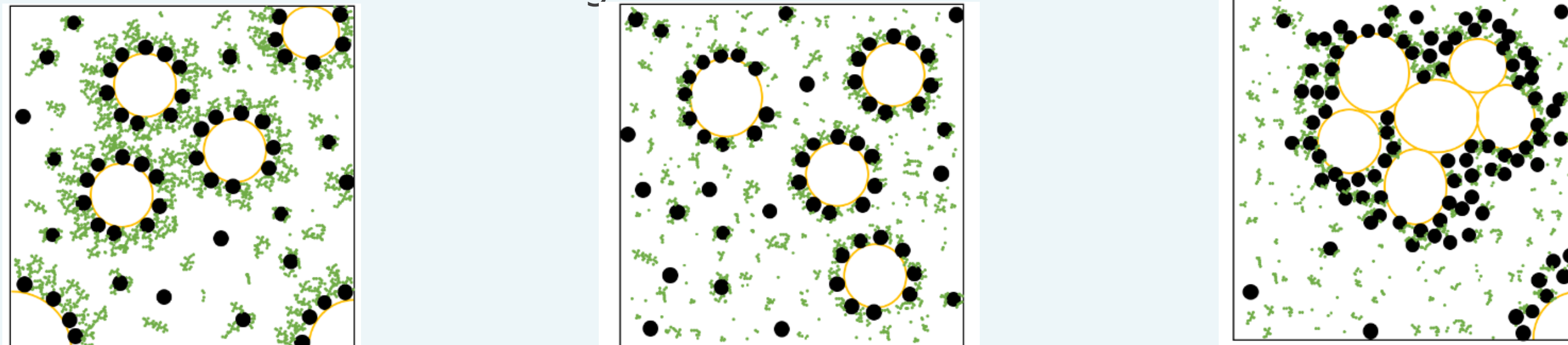
## IN VIVO DIGESTION AND ABSORPTION MODEL



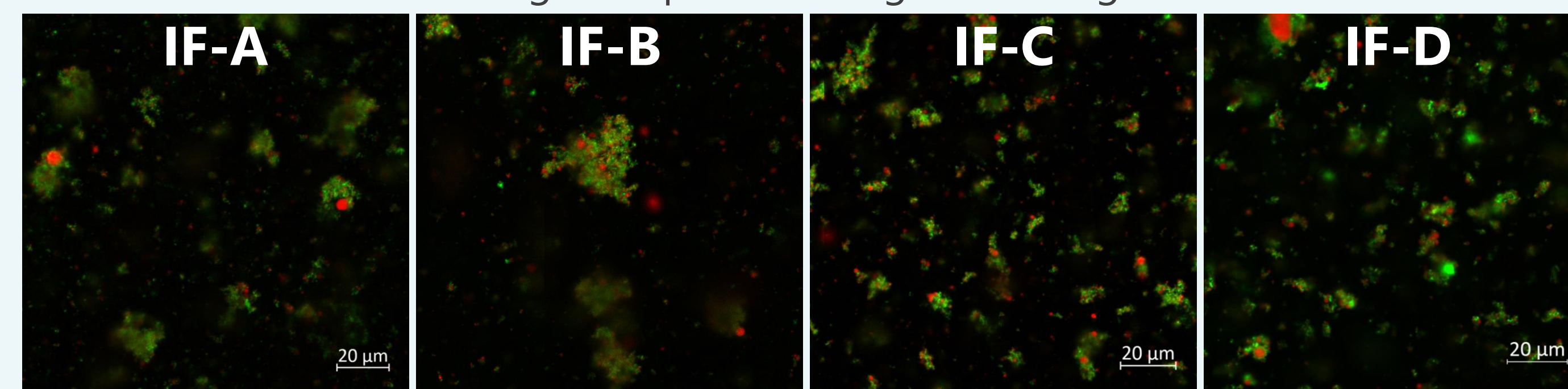
## RESULTS

### STRUCTURE BEFORE AND DURING IN VITRO DIGESTION

Structure of IF before *in vitro* digestion



Structure of IF at 40 min of gastric phase during *in vitro* digestion



#### Structure highlights before digestion :

- **IF A = denatured WPs aggregated** at the interface of casein micelles, themselves **adsorbed at the surface of fat droplets**.
- **IF B & C = mixture** of both **native and denatured/aggregated WPs** in the soluble phase, and with **caseins adsorbed at the surface of fat droplets**.
- **IF D = large aggregates** of **fat droplets, denatured/aggregated WPs and caseins**

#### Structure highlights during *in vitro* digestion :

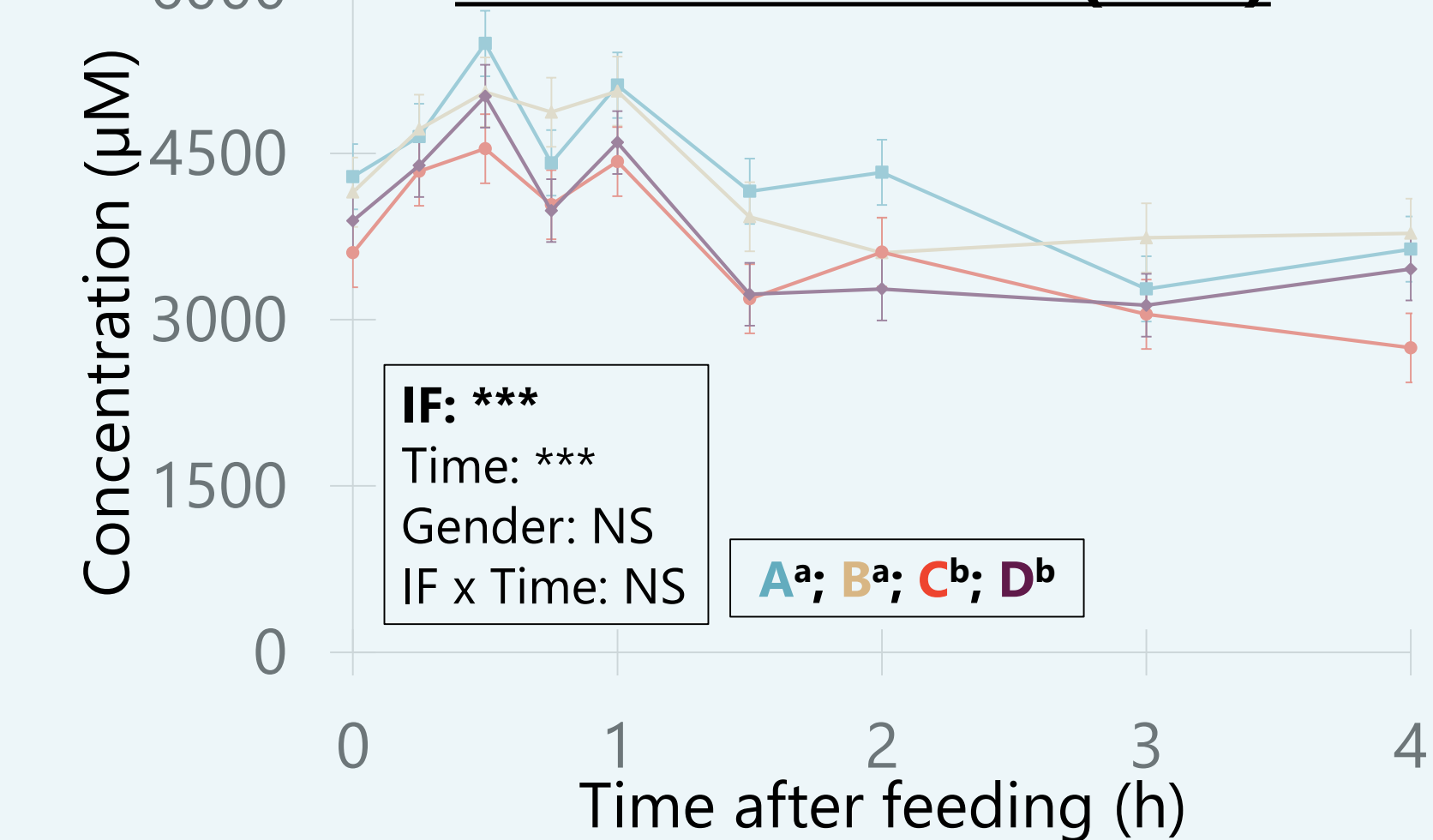
- **Aggregates size** → **IFs-A/-B > IF-C > IF-D**
- **Aggregates size differences** were directly related to the **proteolysis of κ-casein by pepsin** which led to the **rapid aggregation of caseins and fats droplets**

#### Peptide release kinetics :

- **More abundant release of casein-derived peptides at 80 min of gastric phase in IF-D digesta** than in other IFs digestas → Related to the **difference in casein supramolecular organization**
- **More abundant and late release (intestinal phase) of α-lactalbumin and β-lactoglobulin derived peptides** in digestas of **IFs-C and -D** than in digestas of IFs-A and -B → related to the **higher level of native WPs** in IFs-C and -D. (Chauvet et al., 2023)

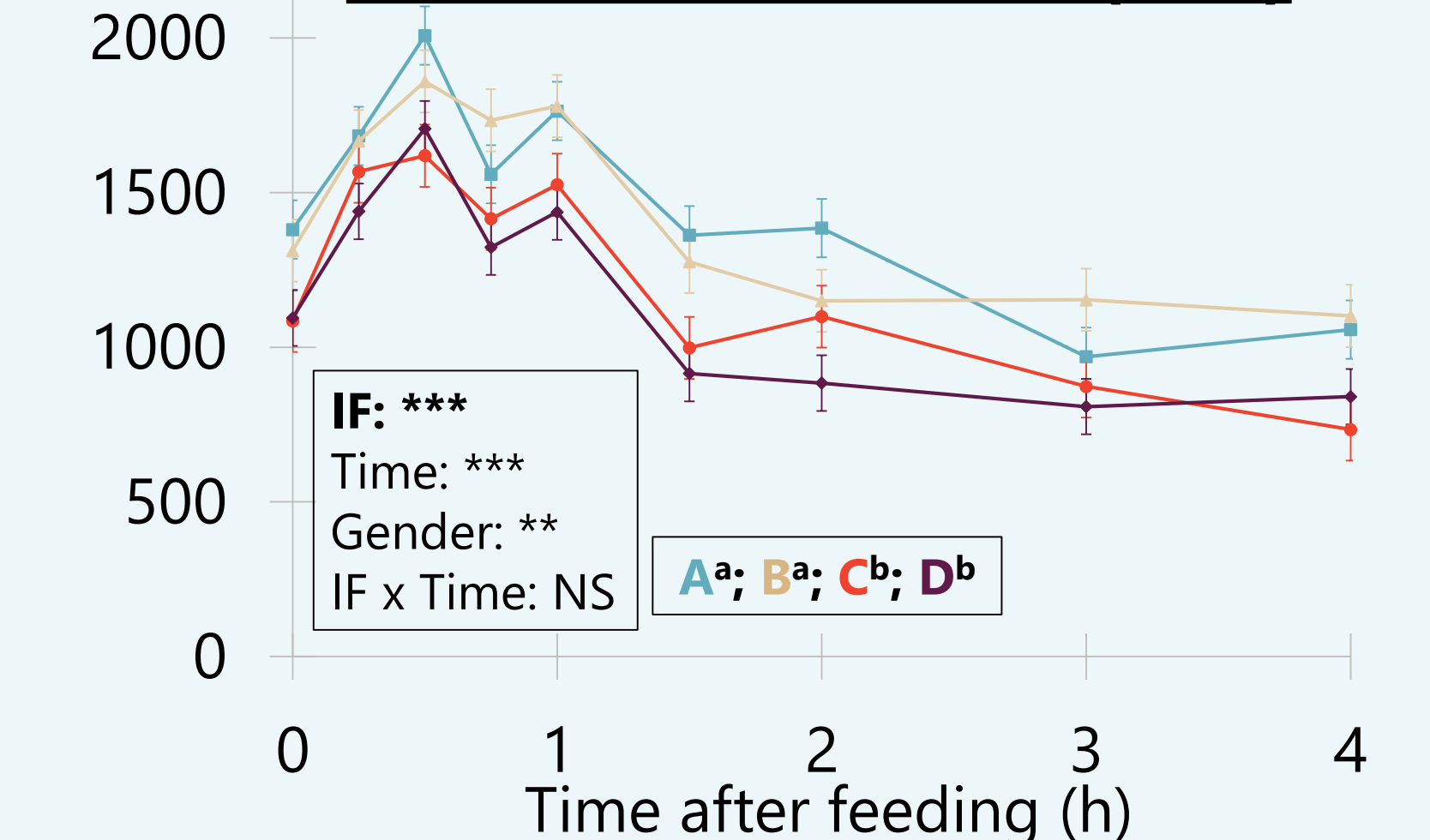
### IN VIVO DIGESTION AND ABSORPTION

#### Total amino acids (TAA)



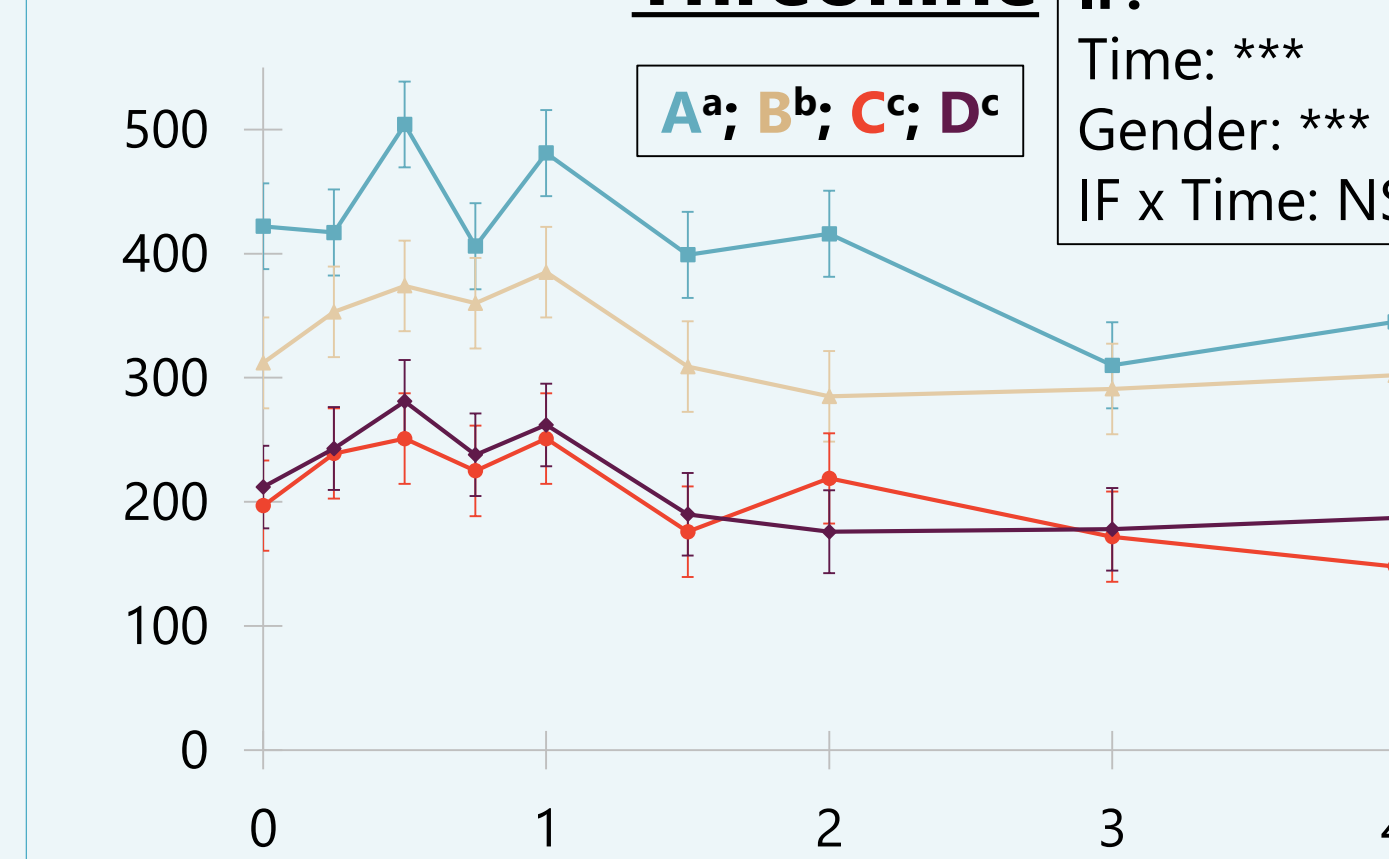
**Plasma TAA concentrations : IFs-A & -B > IFs-C (+17%) & -D (+12%) at both preprandial and postprandial times.**

#### Essential amino acids (EAA)



**Plasma TAA differences are mainly due to the plasma EAA concentrations : IFs-A & -B > IFs-C (+20%) and -D (+26%)**

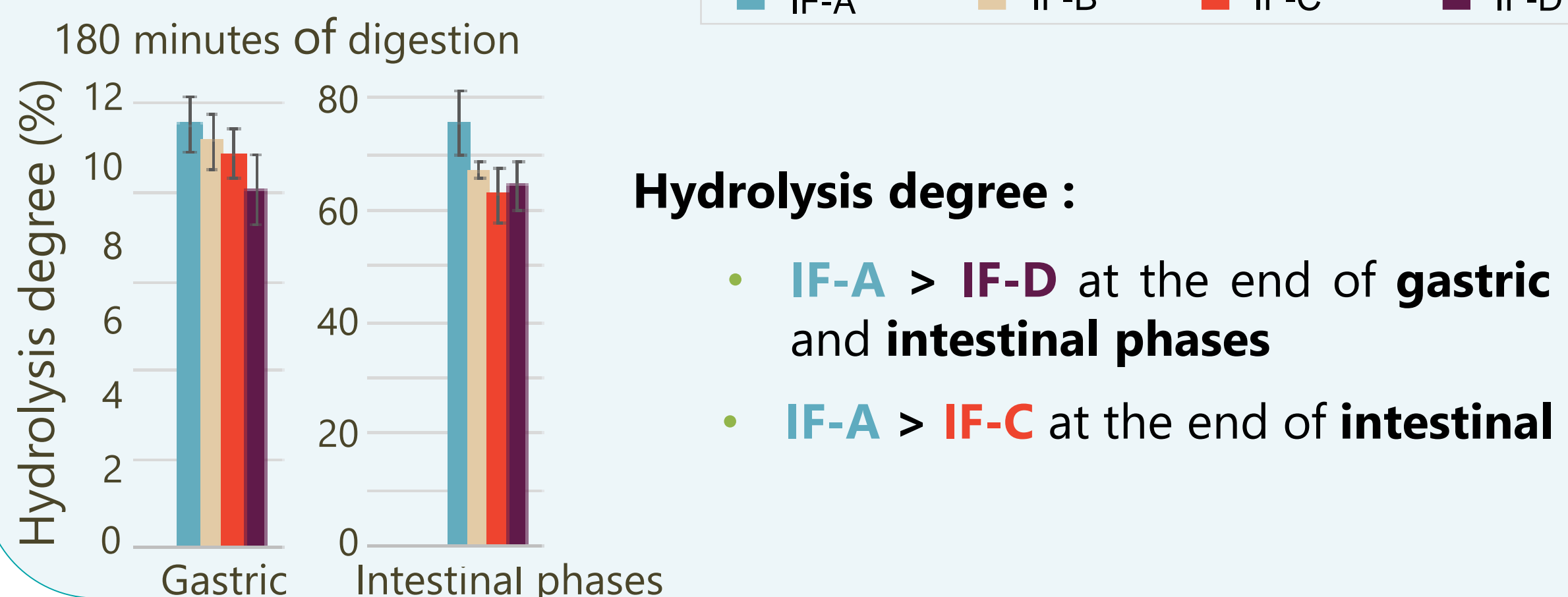
#### Threonine



↑ **dietary Thr level (IFs-A and -B) + limited Thr degradation enzyme capability in piglets = modification of Thr homeostasis**

Modifications since preprandial time = **Modification of AA homeostasis**  
↓  
**Rapid metabolic adaptation** after a 2-day period of IF consumption.

### PROTEOLYSIS



## CONCLUSIONS & PERSPECTIVES

- ↳ **WPs denaturation and casein supramolecular organisation** impact the **emulsion microstructure** within IFs
- ↳ **Proteolysis is favoured** during *in vitro* dynamic digestion when **WPs are more denatured** and **peptide-release kinetics** are modulated by the **casein organisation**

- ↳ The **origin of the WP ingredients** (cheese vs. ideal whey) resulted in the main **differences in plasma AA levels due to the presence or not of GMP**.
- ↳ **Homeostasis of many AAs was modified** after a **short adaptation period** and most of the differences observed preprandially explained the differences observed postprandially.

**THESE RESULTS HIGHLIGHT THE IMPORTANCE OF CONSIDERING THE QUALITY OF THE PROTEIN INGREDIENTS WHEN MANUFACTURING IFs**