Standardized ileal digestibility of amino acids and nitrogen in human milk and infant formula – an in vivo study

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STANDARDISED ILEAL DIGESTIBILITY OF AMINO ACIDS AND NITROGEN IN HUMAN MILK AND INFANT FORMULA – AN IN VIVO STUDY

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INTRODUCTION

- Infant formula (IF) aims to mimic human milk (HM), including its aminogram
- Few *in vivo* data exist on their protein and amino acid (AA) digestibility, particularly regarding tryptophan, an essential amino acid, although essential for IF optimisation
- The standardised a.k.a. true digestibility of Tryptophan is not known

OBJECTIVES

- To determine the standardised ileal digestibility of nitrogen and amino acids from HM vs. IF in a pre-clinical model

METHODOLOGY

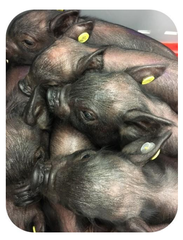
Diets :

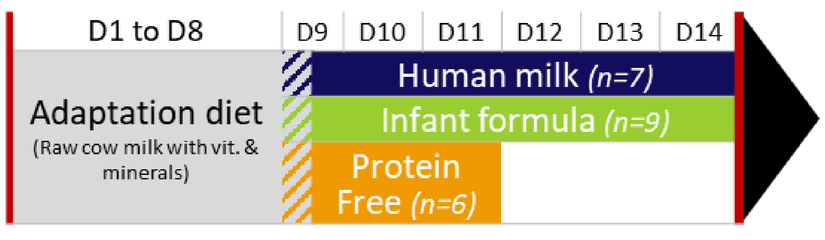
Human Milk: Pool of 50 fresh milk samples, lactation period: 1.8 - 2 months post-delivery, 1.0% total proteins

Infant formula: Standard IF powder rehydrated at 1.4% total proteins (Yu et al. 2021)

Protein-Free Diet: for endogenous N and AA flow measurement

Experimental design :





- 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 30min after last meal
- Undigestible & unabsorbable dietary marker: Co-EDTA at 0.3% dry matter

1. Ileal Digesta collection (last 60 cm) and freeze-drying
2. Amino acid (acid hydrolysis & Cation exchange chromatography) and Tryptophan contents (basic hydrolysis & HPLC, fluorimetry detection), Total Nitrogen (Dumas), Marker (Cobalt, ICP-MS)
3. Standardised digestibility calculation
4. Statistical analysis (Anova, Digestibility~diet+block)

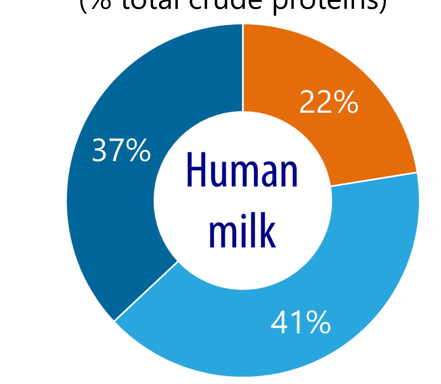
$$\text{Standardised ileal digestibility (\%)} = 100 \times \frac{\text{dietary AA intake} - (\text{AAFL}_{\text{digesta}} - \text{AAFL}_{\text{endogeneous}})}{\text{dietary AA intake}}$$

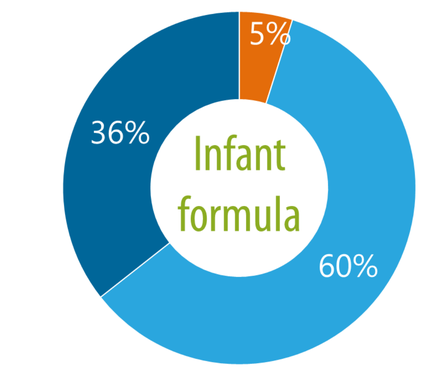
$$\text{AAFL}_{\text{endogenous}} = \frac{\text{AA}_{\text{PF digesta}} \times \text{Marker}_{\text{PF diet}}}{\text{Marker}_{\text{PF digesta}}} \qquad \text{AAFL}_{\text{digesta}} = \frac{\text{AA}_{\text{digesta}} \times \text{Marker}_{\text{diet}}}{\text{Marker}_{\text{digesta}}}$$

AAFL: Amino Acid FLow

RESULTS

Nitrogen fractions of the diets
(% total crude proteins)



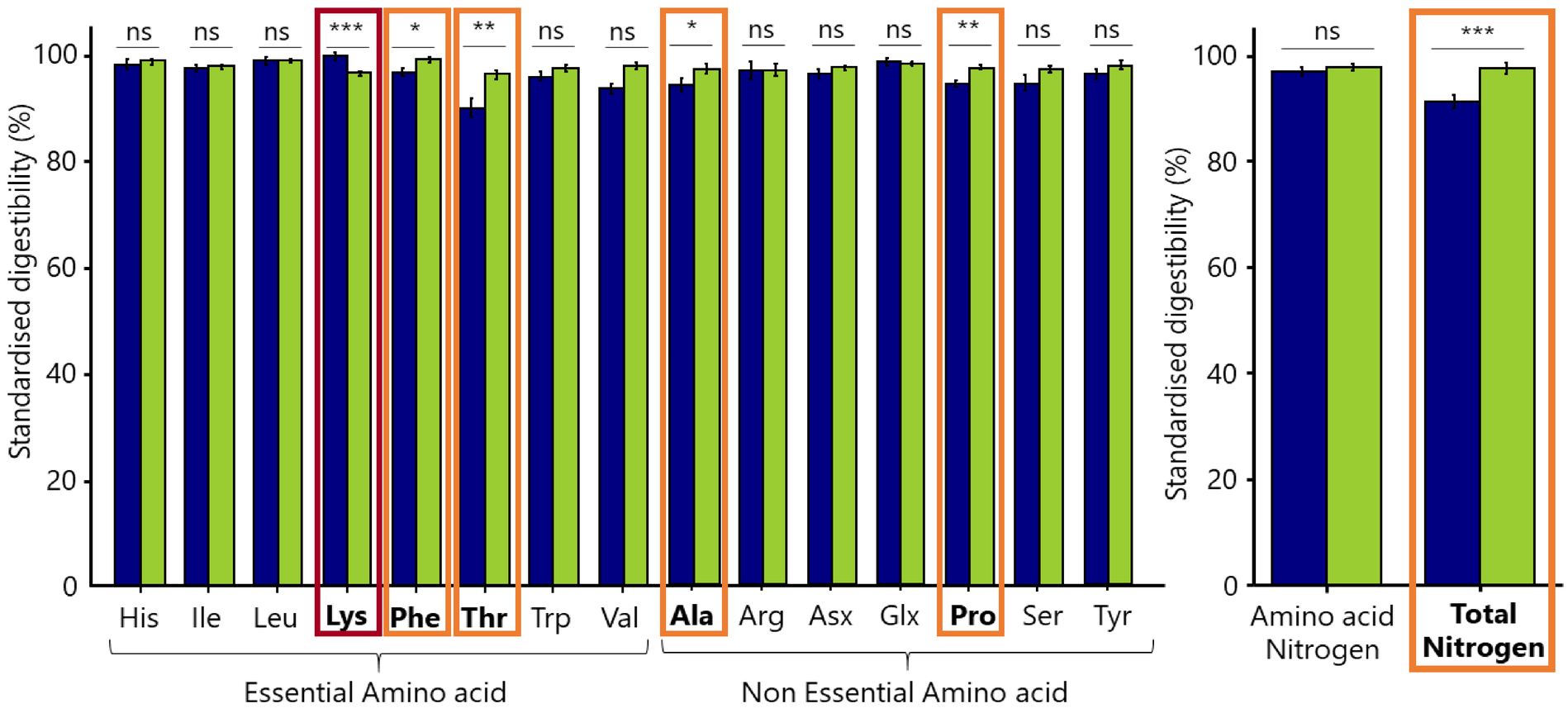


% Non-protein Nitrogen

Protein nitrogen:
% Essential amino acids
% Non-essential amino acids

Human milk Infant formula Higher digestibility in HM Lower digestibility in HM

Standardised digestibility of Amino acid, Amino acid nitrogen and Total nitrogen (%)



Highlights:

- **Tryptophan** standardised digestibility was **similar** between diets with an average value of 96. 3±0.6 %.
- **Lysine** digestibility was significantly **higher in HM** → Lysine reacts with lactose during IF process and forms Maillard reaction products (e.g. CML), which reduces its bioavailability.
- Standardised digestibility was significantly **lower** for **Phenylalanine**, **Threonine**, **Alanine** and **Proline in HM** than IF.
- **Total nitrogen** digestibility was significantly **different** for HM than for IF while **AA nitrogen** was **similar** : this is due to the higher non-nitrogen fraction (4x), partly undigestible/unabsorbable, such as for urea (+89%) and NH_3 (+72%).
- Measured digestibilities agree with the literature data for HM (Darragh et al., 1994) and for IF (Rutherfurd et al., 2006).

CONCLUSION

While IF formulation objective is to best mimic the composition of HM, some discrepancies still exist regarding IF fine protein and AA composition and digestibility. It suggests that some HM component may have physiological role in intestine such as NPN fraction, generally not considered for IF formulation, containing compounds such as urea having bifidogenic properties.

Further investigation will be conducted to unravel the role of the diet on the microbiota-gut-brain axis.

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