**Supplementary material 1.1.** Characteristics of the enzymes and bile salts used for the simulated *in vitro* gastrointestinal digestion as described by the INFOGEST protocol.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Enzyme | Commercial name | Commercial reference | Batch number | Experimental activity (U/mg) |
| Pepsin | Pepsin from porcine gastric mucosa | P7012 | SLBW6530 | 2639.8 |
| Bile | Bile extract porcine | B8631 | SLCC9272 | 1.3 (mmol/g) |
| Pancreatin | Pancreatin from porcine pancreas | P7545 | SLCD7175 | 4.45\* |

Enzymes and bile were purchased from Sigma-Aldrich (Merck, Zug, Switzerland). The activity of all enzymes used for digestion assays was previously measured according to INFOGEST protocol guidelines (Brodkorb et al., 2019).

\*Pancreatin activity was expressed as trypsin activity using the synthetic substrate p-toluene-sulfonyl-L-arginine methyl ester (TAME) as described by Brodkorb et al. (2019).

**Supplementary material 1.2.** Chemical composition of the gastrointestinal fluids used for simulated *in vitro* gastrointestinal digestion following the INFOGEST protocol. Values in the table correspond to final concentrations of each reagent, expressed as mM.

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | SSF | SGF | SIF |
| KCl | 15.1 | 6.9 | 6.8 |
| KH2PO3 | 3.7 | 0.9 | 0.8 |
| NaHCO3 | 13.6 | 25 | 85 |
| NaCl | - | 47.2 | 38.4 |
| MgCl2(H2O)6 | 0.15 | 0.12 | 0.33 |
| (NH4)2CO3 | 0.06 | 0.5 | - |
| pH final | 7 | 3 | 7 |

SSF: simulated salivary fluid; SGF: simulated gastric fluid; SIF: simulated intestinal fluid.

**Supplementary material 1.3.** Calculation of *in vitro* digestibility and DIAAR (Sousa et al., 2023).

Total protein *in vitro* digestibility was calculated using the sum of individual amino acids (UHPLC) or the amount of primary amines (OPA method) analysed in the supernatants and pellets after *in vitro* digestion. The digestibility of individual amino acids, the digestible indispensable amino acid ratio (DIAAR) and the digestible indispensable amino acid score (DIAAS) were calculated based on individual amino acids (UHPLC).

Calculation of digestibility was performed with the total amount of amino acids, primary amines, or individual amino acids in food supernatant (Fs), cookie supernatant (Cs), food pellet (Fp) and cookie pellet (Cp), as follows:

$$in vitro digestibility \left(\%\right)= \frac{(Fs-Cs)}{\left(Fs-Cs\right)+max⁡(0;Fp-Cp)}×100$$

*In vitro* DIAAR is the result from the relation between the indispensable amino acid (IAA) content of food (mg IAA *per* g protein) relative to its digestibility (%), and the daily reference requirements (mg IAA *per* g protein) based on specifications by FAO (2013) for infant, child and adult nutrition. DIAAR is calculated following this formula:

$$in vitro DIAAR \left(\%\right)= \frac{^{mg IAA}/\_{g protein in food}×IAA in vitro digestibility}{daily requirements of ^{mg IAA}/\_{g protein}}$$

*In vitro* DIAAS is the lowest DIAAR of a sample, and the IAA corresponding to the DIAAS is considered the limiting amino acid of the food sample.

**Supplementary material 1.4.** Digestibility of individual mare milk amino acids expressed as percentage (%). Numbers above the bars represent the mean digestibility % for each amino acid. Error bars represent standard deviation. ALA: alanine, ARG: arginine, ASP: aspartic acid, CYS: cysteine, GLU: glutamic acid, GLY: glycine, HIS: histidine, ILE: isoleucine, LEU: leucine, LYS: lysine, MET: methionine, PHE: phenylalanine, PRO: proline, SER: serine, THR: threonine, TRP: tryptophan, TYR: tyrosine, VAL: valine.

