

Validation of an *in vitro* digestion model

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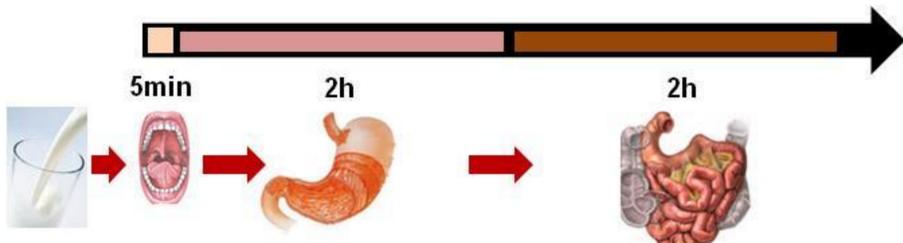


INTRODUCTION

Bovine milk contains many essential and bioactive nutrients. To enable their metabolic action in the body, these nutrients have to be digested and transported through the intestinal barrier. In this study a three-steps *in vitro* digestion model was developed that mimics the digestion process taking place in humans. The model was validated by following the composition of the macronutrients in pasteurized whole milk during the digestion process.

METHODS

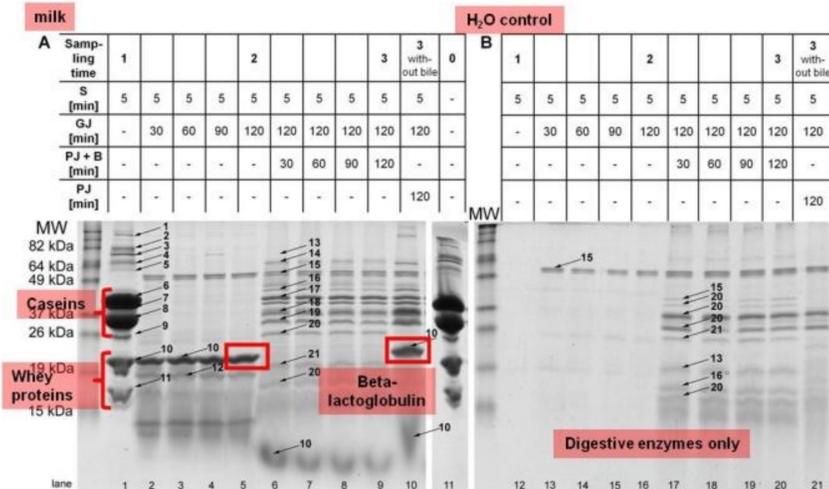
In vitro digestion model



Analysis of macronutrients: gel electrophoresis; HPLC; LC-MS; GC-MS; size-exclusion chromatography; enzymatic assays

RESULTS

Protein degradation (gel electrophoresis)



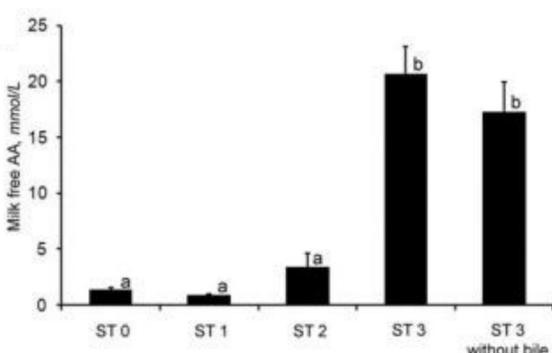
➤ Caseins and whey proteins are differentially digested during the 3 steps

Detection of free amino acids, dipeptides and tripeptides (OPA-method)

Digestion stage	mmol glutamate equivalents / L milk
Undigested milk	3.37 ± 1.52
Complete digestion	112 ± 5.46
Digestion without bile	90.0 ± 5.24

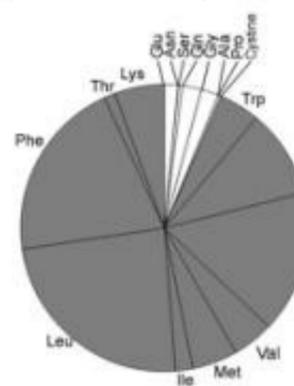
➤ ~54% of the milk proteins are released in form of free amino acids, dipeptides or tripeptides

Free amino acid determination (HPLC)

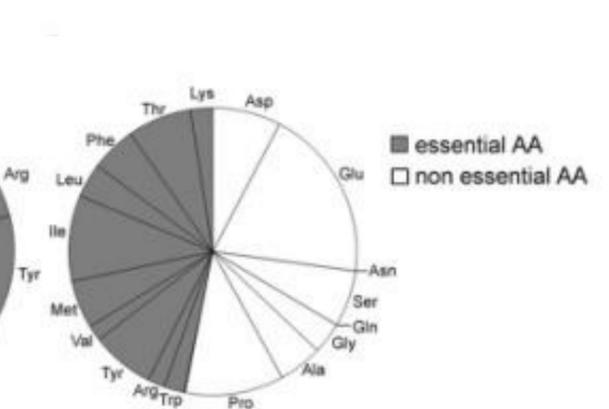


➤ ~10% of the milk protein is released in form of free amino acids after complete digestion

Free amino acids released after complete digestion

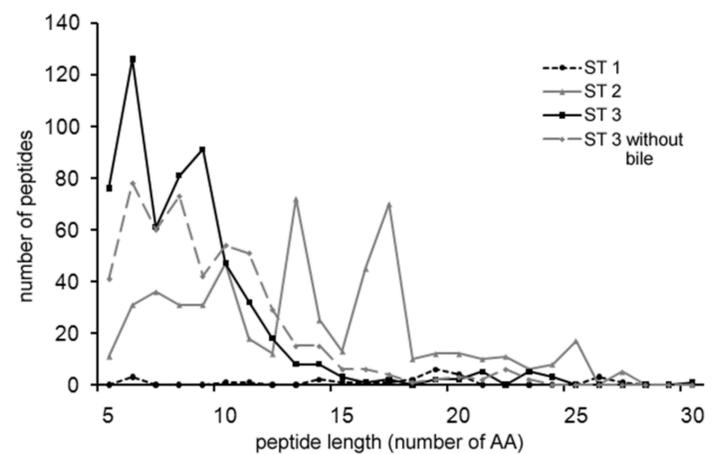


Amino acid composition of milk



➤ The free amino acids that are released during digestion are mainly essential (~94%)

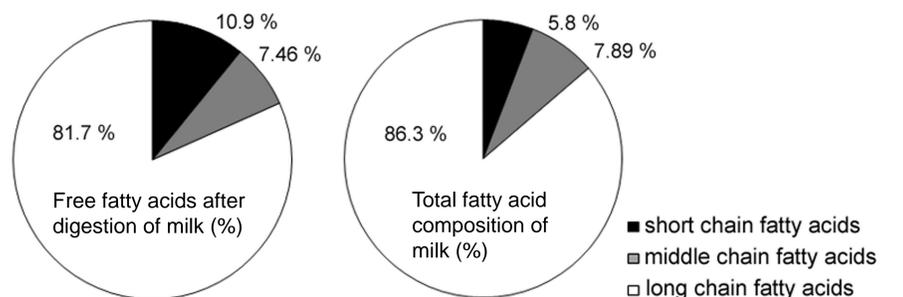
Peptide length detection (LC-MS)



➤ Proteins are degraded into amino acid and small peptides

- 47% are free amino acids, dipeptides or tripeptides
- 8.7% are free amino acids
- 93.6% of the free amino acids are essential amino acids
- The average peptide size is 3-6 amino acids

Fatty acid release during digestion (GC-MS)



➤ ~ 100% of the milk fat is released in form of free fatty acids and monoacylglycerides

CONCLUSIONS

The composition of the digested macronutrients in milk is in agreement with human physiological values
The digested products can be applied onto the intestinal co-culture model (NutriChip) to study nutrient absorption and immune-modulation