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Agroscope

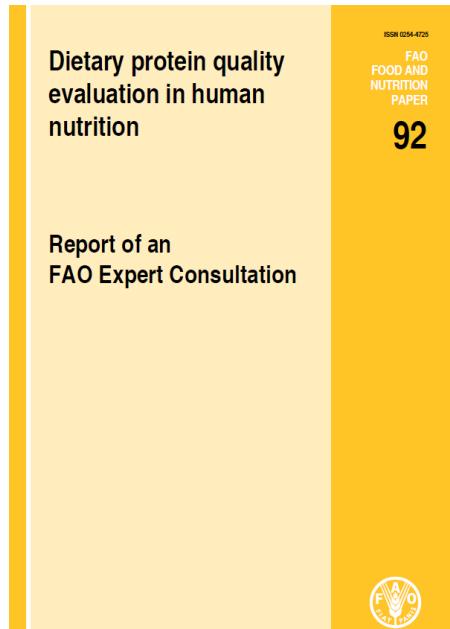
Standardization of *in vitro* digestibility and DIAAS method based on the static INFOGEST protocol

**Raquel Sousa, Isidra Recio, Paul Moughan, Suzanne
Hodgkinson, Reto Portmann, and Lotti Egger**

ICFD 2022, Cork, 3-5th May



Dietary protein quality evaluation by FAO



Ileal digestibility

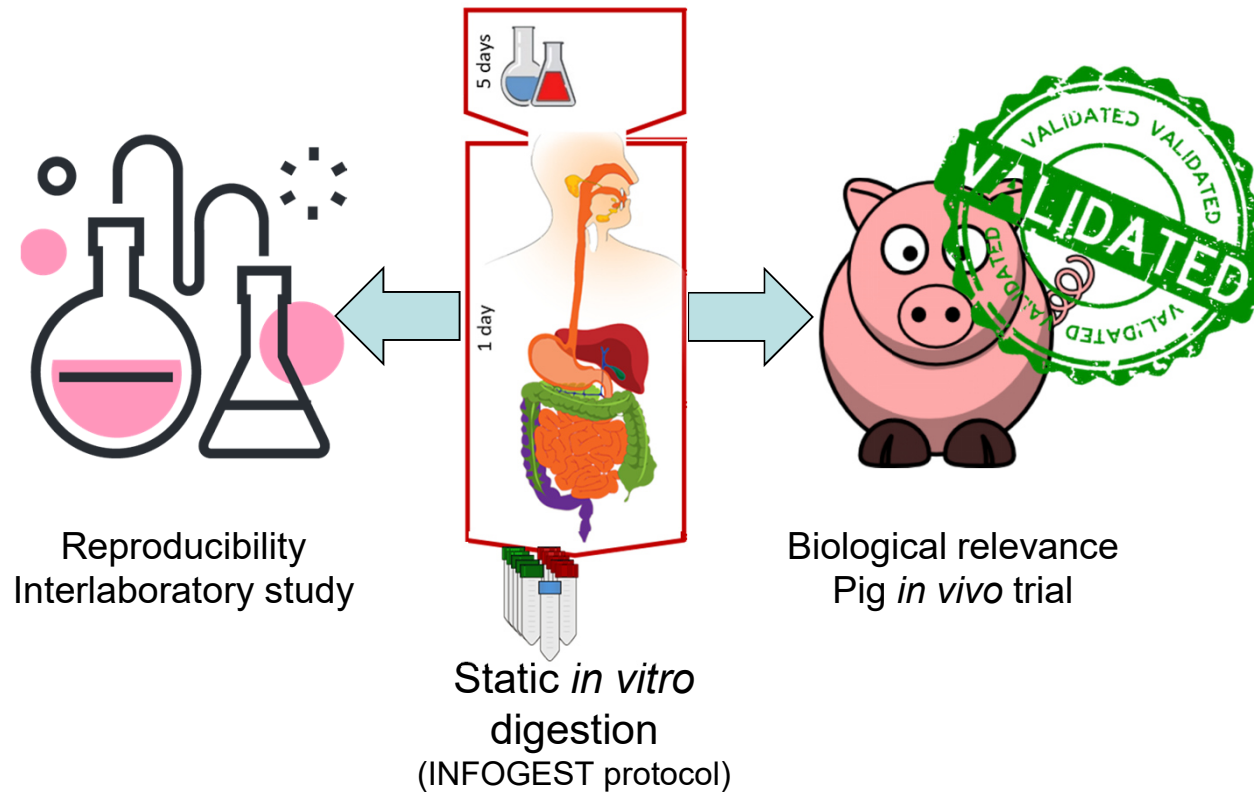
1. Further determine true ileal digestibility of protein and amino acids in a wider range of foods and determine the ileal digestible tryptophan content of human milk.
2. Develop non-invasive accurate methods to determine or predict true ileal dietary protein and amino acid digestibility in humans based on identified biomarkers.
3. Validate the use of animal model data (including providing more robust inter-species prediction equations for true ileal amino acid digestibility) to quantify ileal digestibility in humans, including relating digestibility to functional outcomes.
4. Determine more fully the role of the small intestinal and colonic microflora on ileal amino acid digestibility values.
5. Develop new bioavailability assays such as the reactive lysine assay, for other amino acids.
6. Develop and validate *in vitro* methods for predicting amino acid digestibility and bioavailability in humans.

DIAAS % = 100 x *lowest value* ["Digestible IAA reference ratio" for a given amino acid scoring pattern].

Note that the main difference between DIAAS and PDCAAS is that true ileal amino acid digestibility for the dietary indispensable amino acids is used rather than a single faecal crude protein digestibility value.



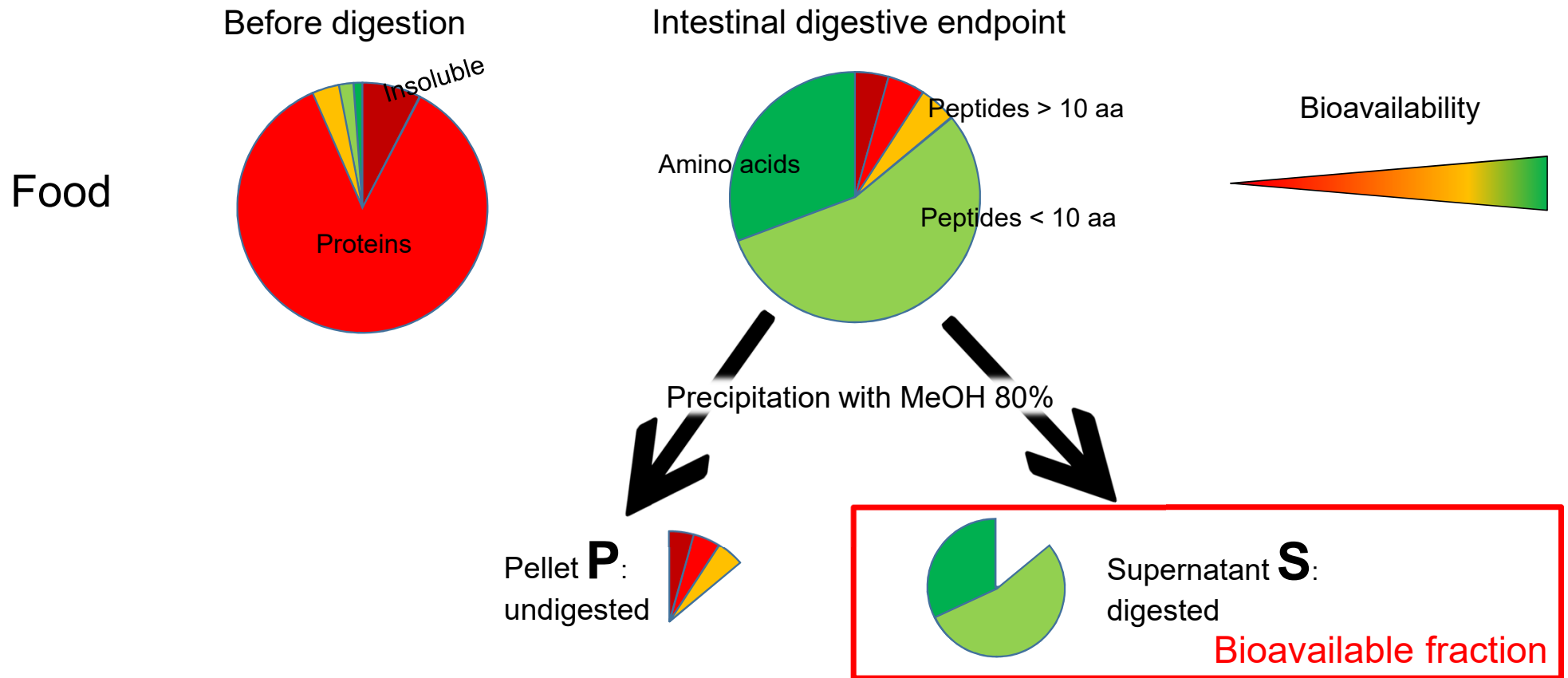
Validation of *in vitro* results with *in vivo* data



→ *in vitro* protein hydrolysis proved to be a good approximation to the *in vivo* situation

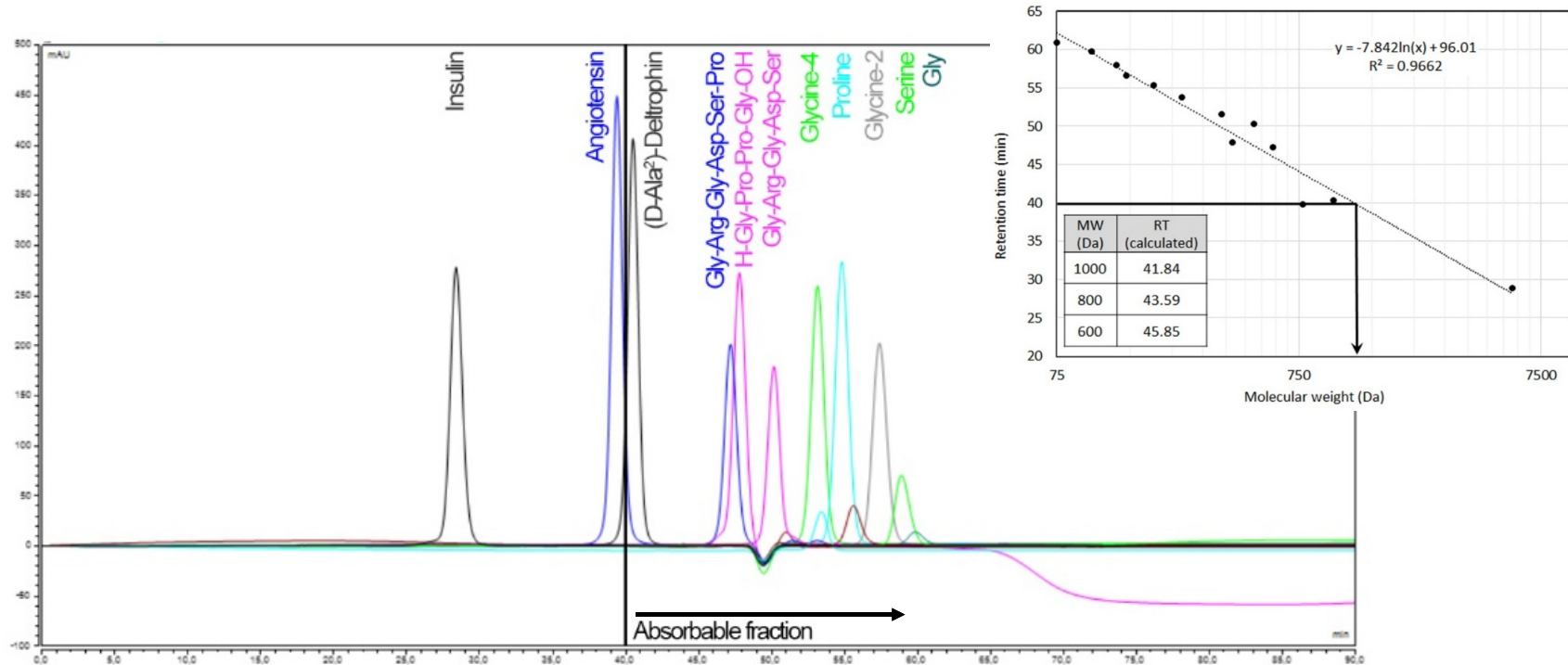


Determination of *in vitro* digestibility





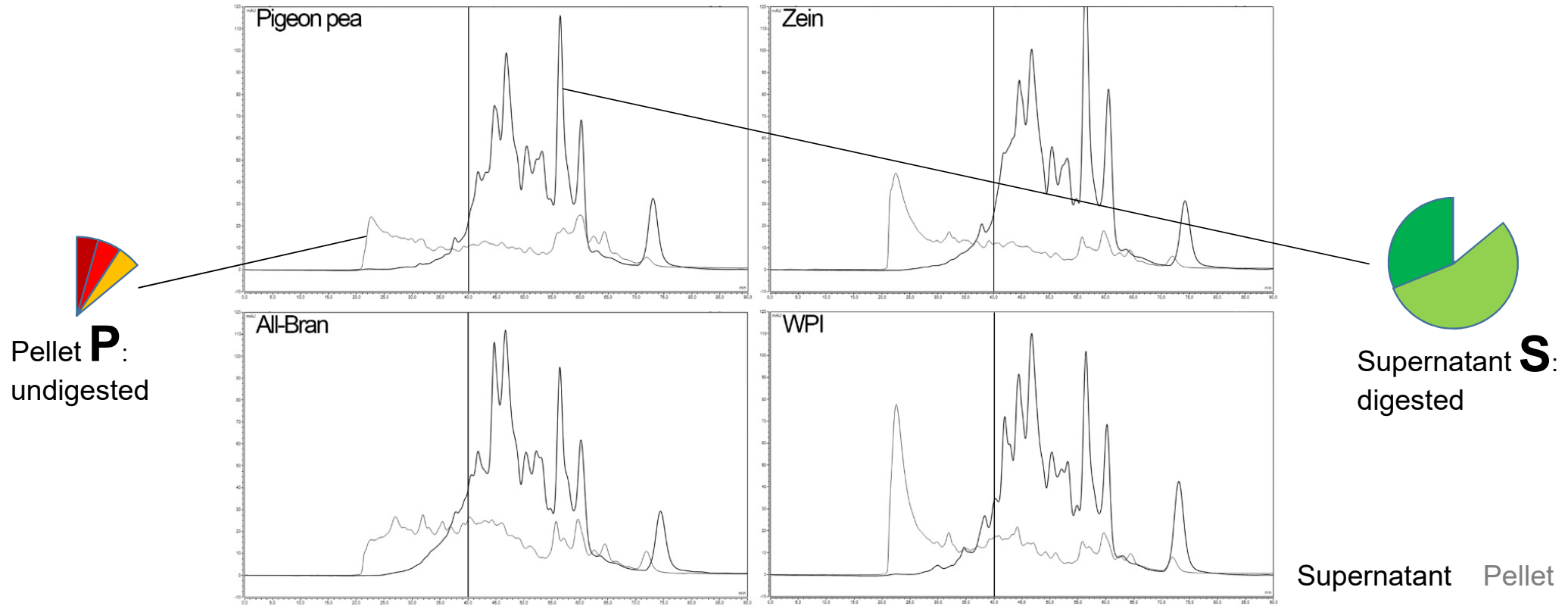
Size exclusion chromatography (SEC)



- Size distribution in samples- heavier molecules elute first, lighter molecules elute later
- Cut-off set up at 40min \approx 1000 Da- peptides between 8 to 10 aa



Precipitation with 80 % MeOH



→ Precipitation separates efficiently bioavailable from non-bioavailable components



The different analytical endpoints

IVD intestinal endpoint	Tested food	pellet supernatant	F_S	Total digestibility		<i>In vitro</i> DIAAS	
				TN	R-NH ₂	TAA	
	MeOH Precipitation 80%, -20° C, 1h	↓	pellet supernatant	F_P	Supernatant		
				Kjeldahl	Hydrolysis 6 N HCl, 110°C, 15 h OPA	HPLC	
	Protein-free cookie		pellet supernatant	C_S	Pellet		
				Kjeldahl	Hydrolysis 6 N HCl, 110°C, 15 h OPA	HPLC	
	Enzyme blank		pellet supernatant	C_P	Calculation		
				$in\ vitro\ digestibility[\%] = \frac{F_S - C_S}{(F_S - C_S) + \max(0; F_P - C_P)} \times 100$			

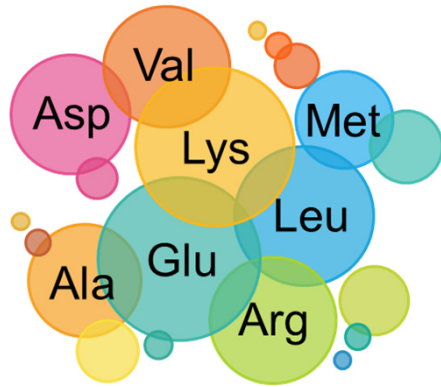
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Digestible indispensable amino acid score (DIAAS)

Amino acid quantification in the food



mg indispensable amino acid per g food protein



× *in vitro* Digestibility_{Lys}

mg digested indispensable amino acid per g food protein (DIAA_{measured})



mg amino acid per g reference protein (DIAA_{reference})

Recommended amino acid scoring patterns for infants, children and older children, adolescents and adults

Age Group	His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
	<i>scoring pattern mg/g protein requirement</i>								
Infant (birth to 6 months) ¹	21	55	96	69	33	94	44	17	55
Child (6 months to 3 year) ²	20	32	66	57	27	52	31	8.5	43
Older child, adolescent, adult ³	16	30	61	48	23	41	25	6.6	40

¹ Infant is based on the gross amino acid content of human milk from Table 4.

² Child group is from the 6 month (0.5 y) values from Table 3.

³ Older child, adolescent, adult group is from the 3-10 y values from Table 3.

FAO: Dietary protein quality evaluation in human nutrition (ISBN 978-92-5-107417-6)

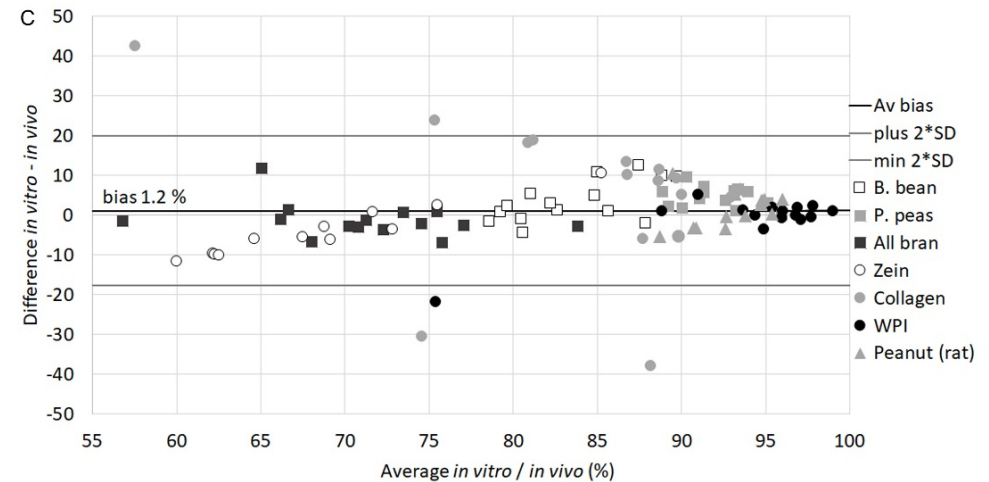
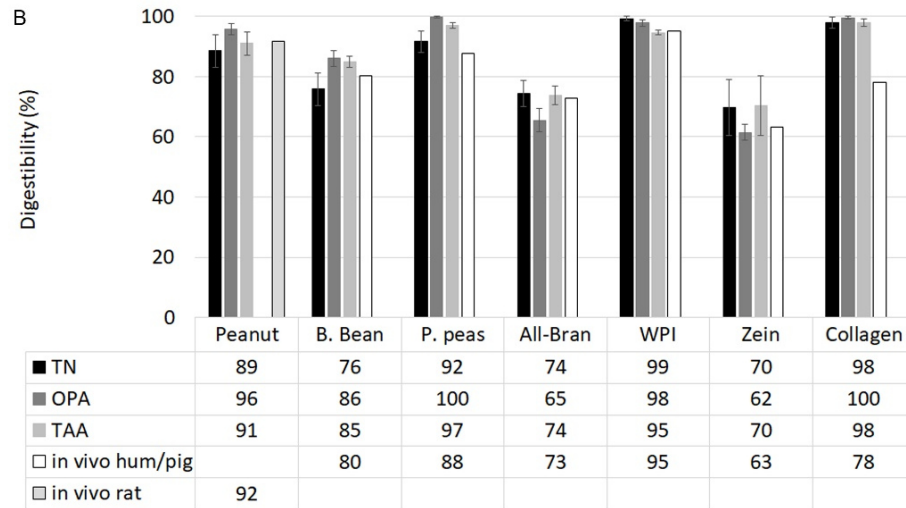
DIAAR: Digestible indispensable amino acid **ratio**

DIAAS: Digestible indispensable amino acid **score** = lowest DIAAR

$$DIAAR = \frac{DIAA_{measured}}{DIAA_{reference}} \times 100$$



In vitro and in vivo digestibility comparison



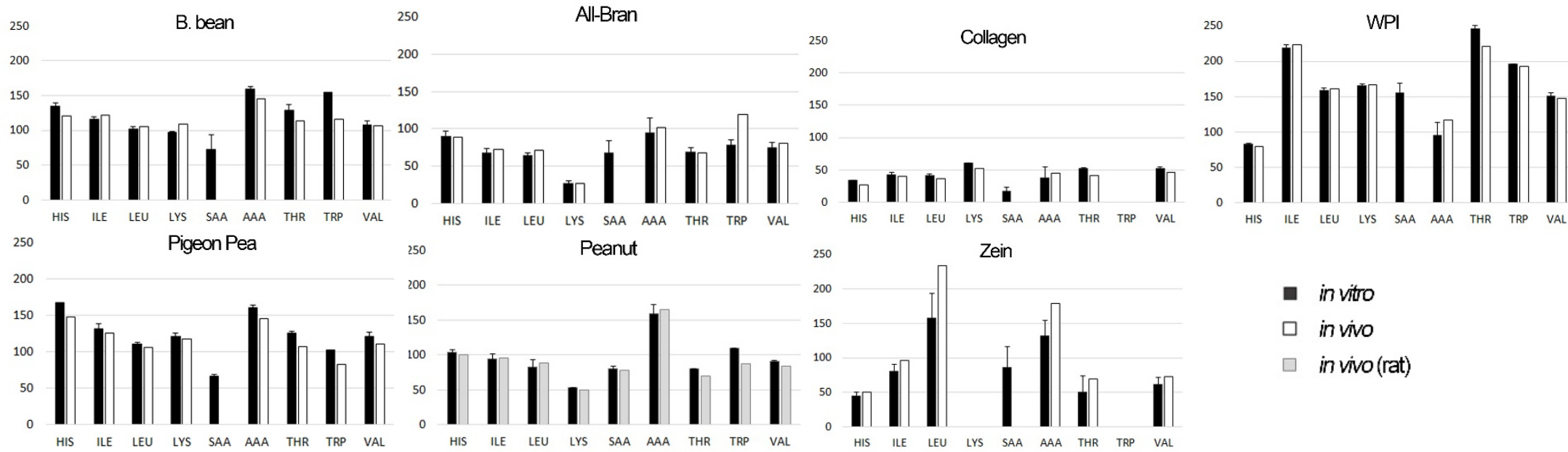
→ *in vitro* values seem to be slightly higher than *in vivo*

→ In general, the three methods give similar results for 7 tested substrates (PROTEOS)

→ Average bias 1.2 %



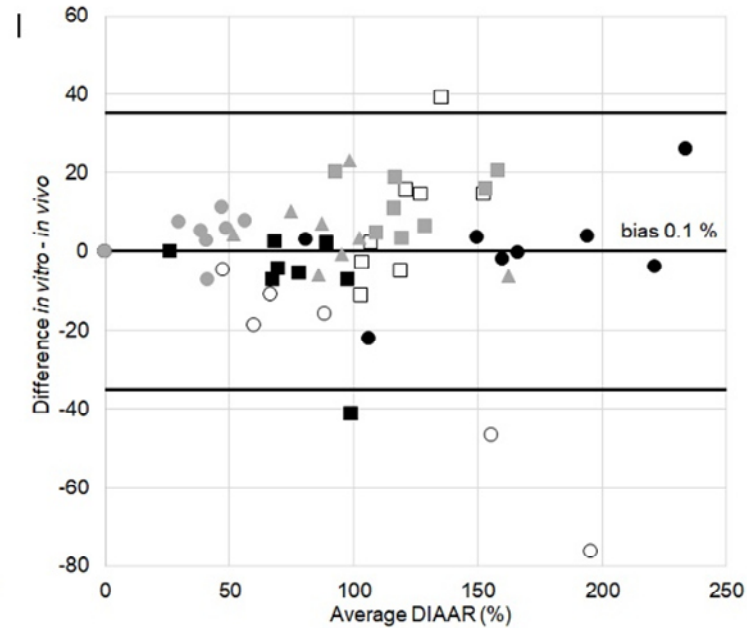
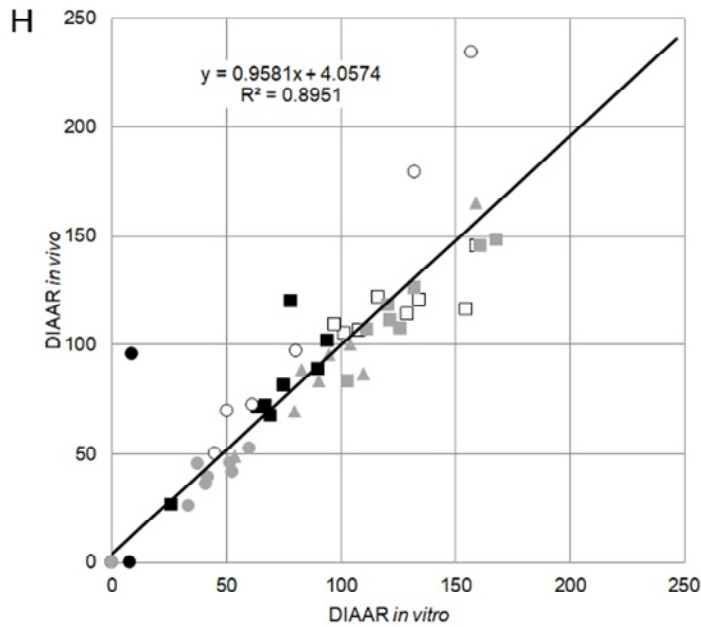
In vitro DIAAR and *in vivo* DIAAR comparison



→ In general, the three methods give similar results for 7 tested substrates (PROTEOS)



In vitro DIAAR and *in vivo* DIAAR correlation

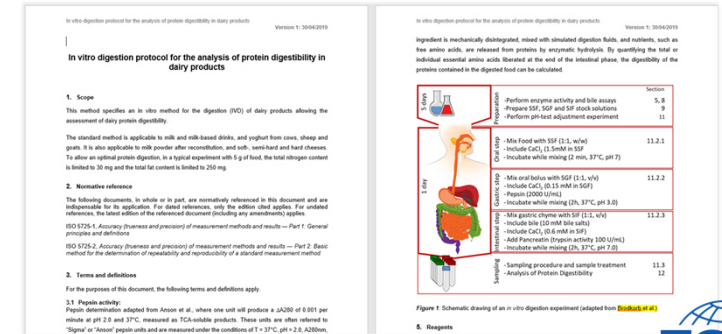


→ Very good correlation between the two methods (correlation slope= 0.96)

→ Average bias between *in vitro* and *in vivo*: 0.1 %



Ringtrial on the use of INFOGEST protocol to determine *in vitro* digestibility in dairy products



Goals:

- Test the robustness, repeatability and reproducibility of the protocol
- Improve and clarify the protocol draft

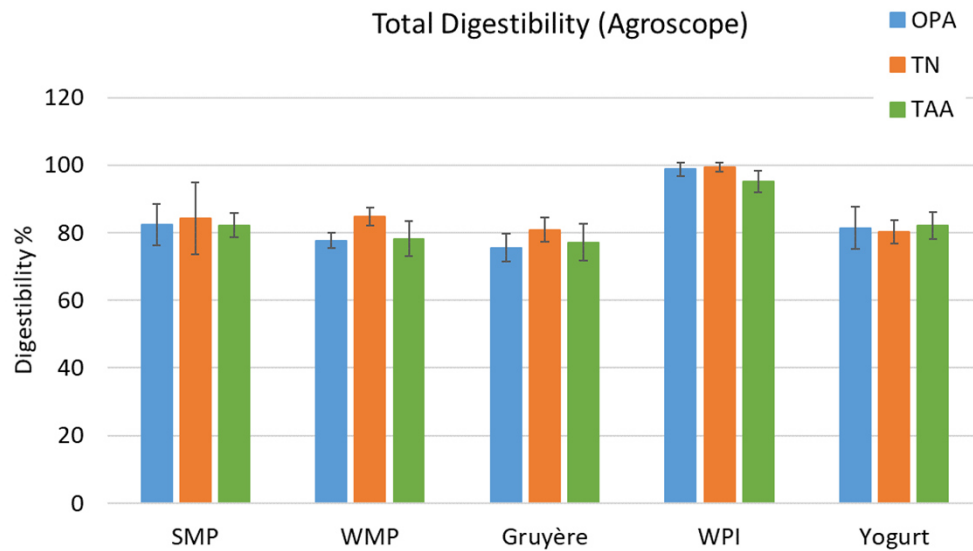
→ 32 labs - 18 different countries | 4 continents

→ Tasks: assay enzyme activities, digest 5 substrates and 1 protein-free cookie in triplicates, calculate digestibility for TN and OPA, SDS-PAGE, and send 1 set of digesta for TAA analysis

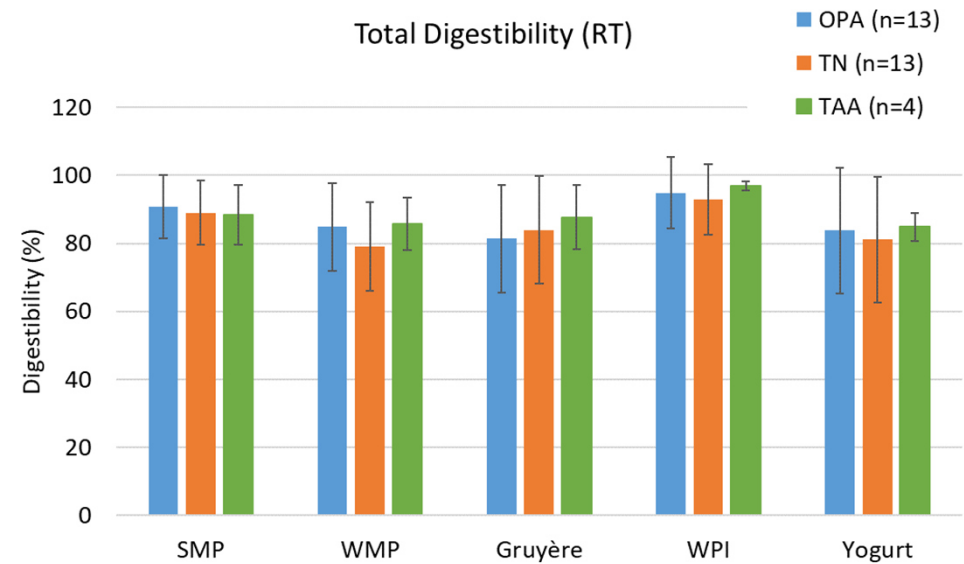
→ Received so far: 20 different labs (results and samples)



Ringtrial: total digestibility



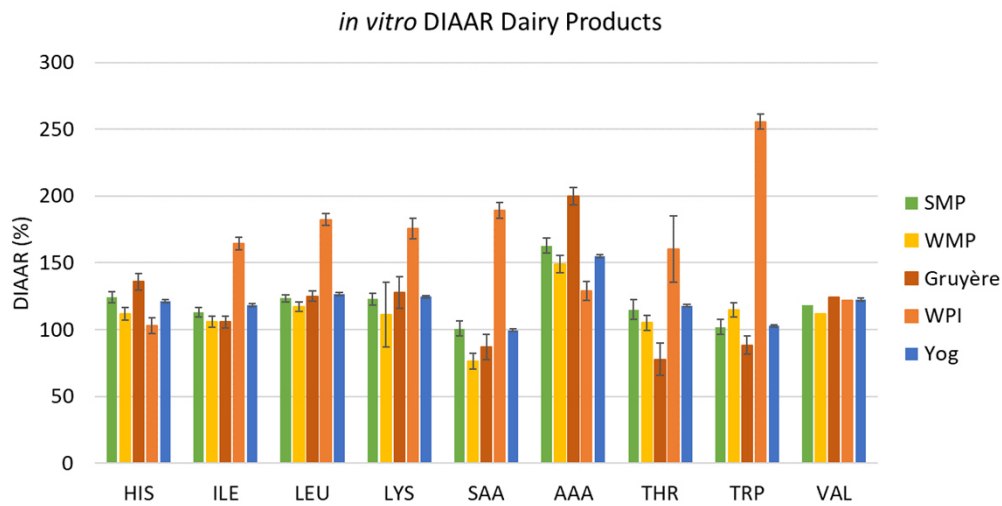
Results from 1 Lab, 2 persons, 6 repetitions



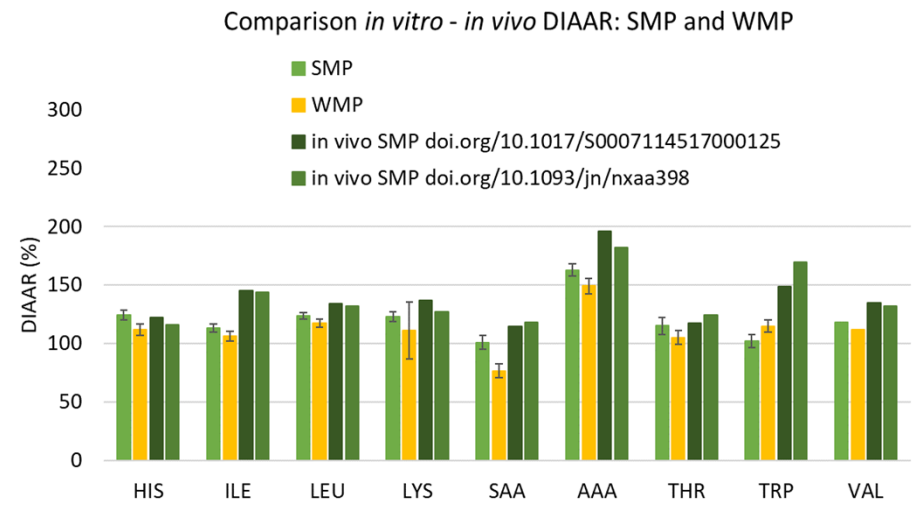
Results from RT labs, each lab 3 repetitions

→ In general, the three methods give similar results for the tested dairy products at the intra-laboratory and at the inter-laboratory level

🇨🇭 Calculation of *in vitro* DIAAR and comparability to *in vivo* data



Results from 1 lab, 3 replicates



Results from 1 lab, comparison with literature

→ Calculation of *in vitro* DIAAR/DIAAS values based on TAA analysis

→ Comparison between *in vivo* data for skim milk powder (SMP)

Conclusions

- *in vitro* method provides a good estimation of the *in vivo* situation for total digestibility, digestibility of individual amino acids and DIAAR.
- Comparison between *in vivo* and *in vitro* approaches was so far tested with seven different protein sources. In the future the method has to be validated with more protein sources.
- The first results of the ringtrial show comparable results between labs.
- Reproducibility of the method can be optimized by clarifying and improving the draft protocol.



Ringtrial: A BIG thank to all the participants

Spain: Isidra Recio, Marta Santos Hernández, Felipe González, Patricia Ruas-Madiedo, Lorena Ruiz, Raquel Olias, Cristina Delgado-Andrade, Ruben Lopez Nicolas

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Thank you for your attention

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