

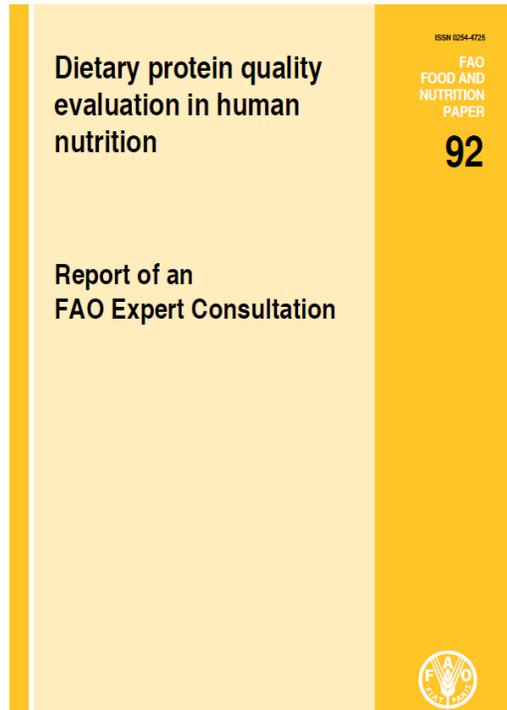


Protein digestibility and DIAAS values: *in vitro* method using the harmonized INFOGEST protocol validated towards *in vivo* data

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Dietary protein quality evaluation by FAO



Ileal digestibility

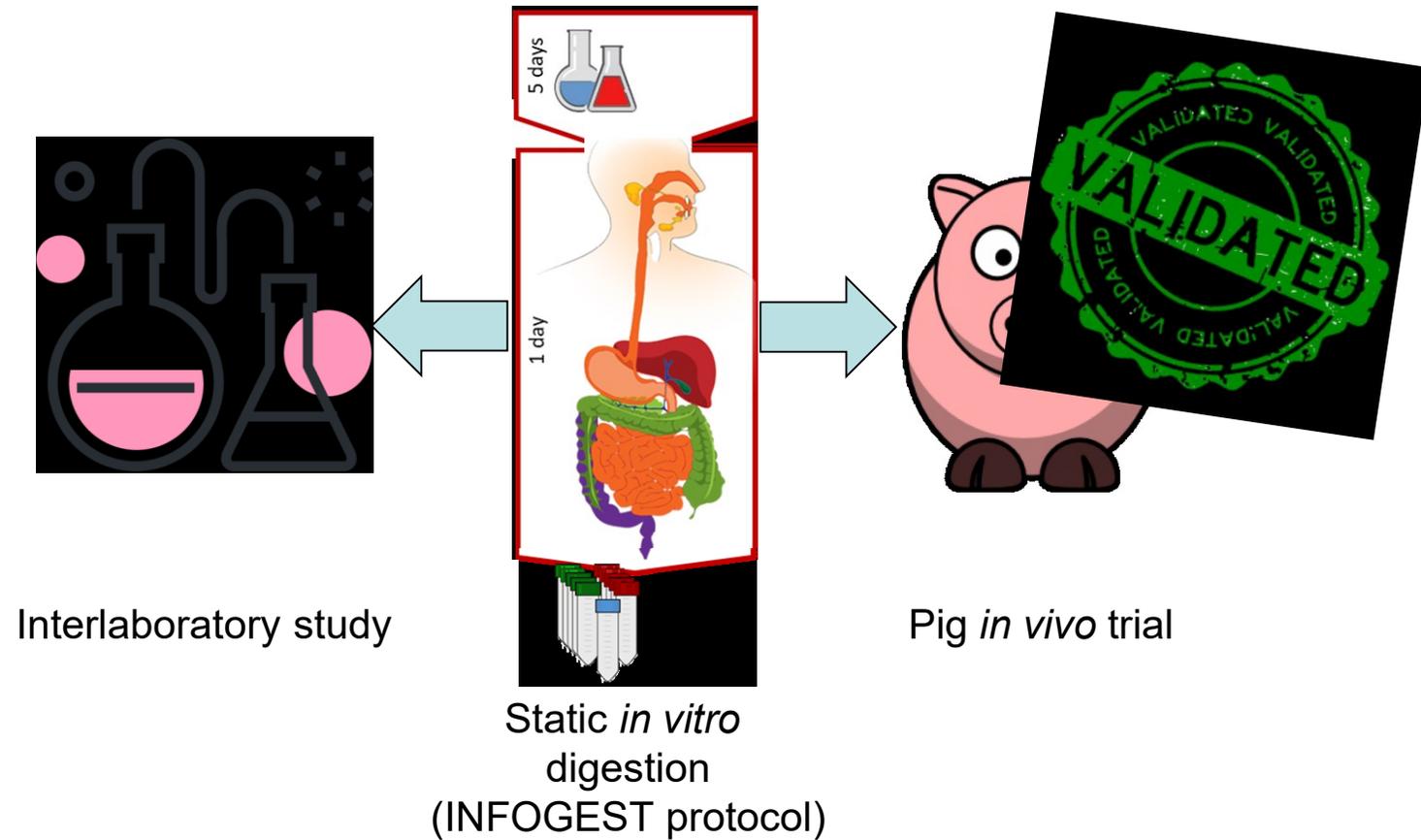
1. Further determine true ileal digestibility of protein and amino acids in a wider range of foods and determine the ileal digestible tryptophan content of human milk.
2. Develop non-invasive accurate methods to determine or predict true ileal dietary protein and amino acid digestibility in humans based 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 % = $100 \times \text{lowest value}$ ["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.



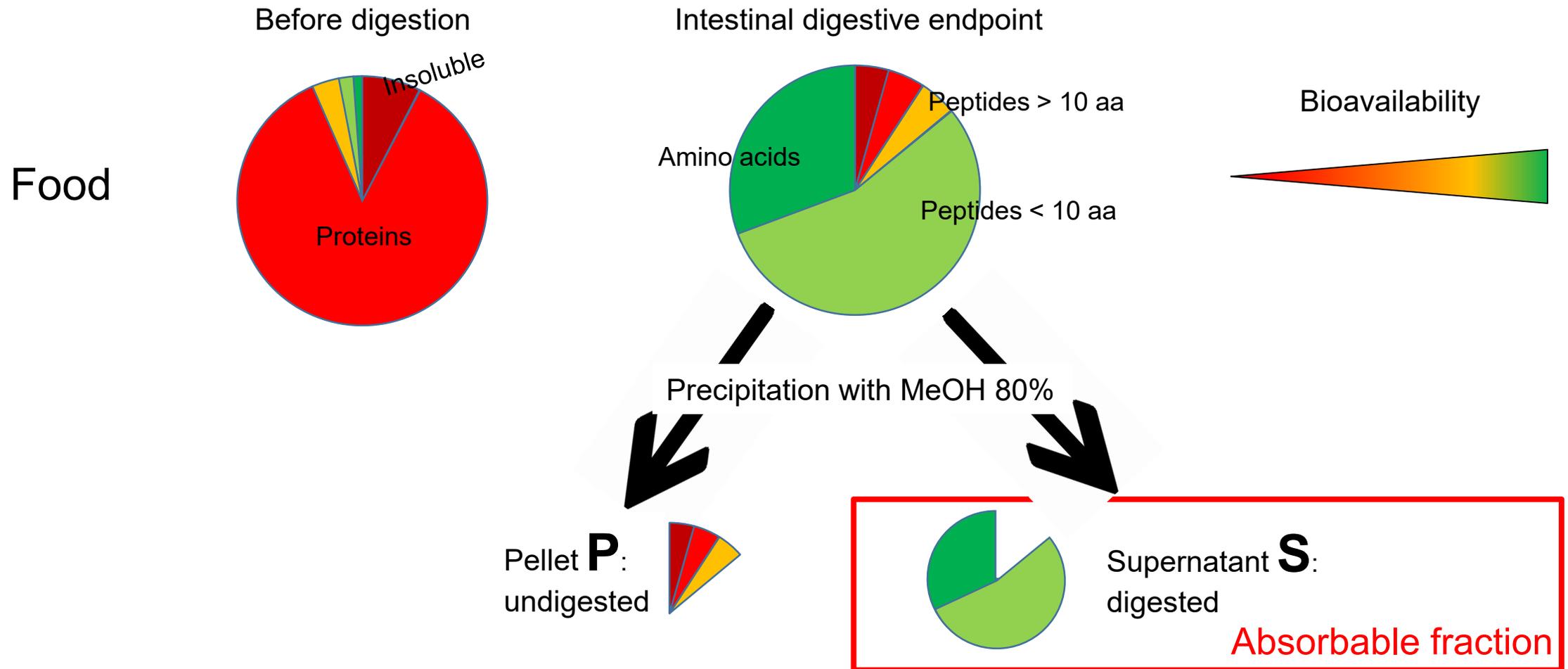
Validation of *in vitro* results with *in vivo* data



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

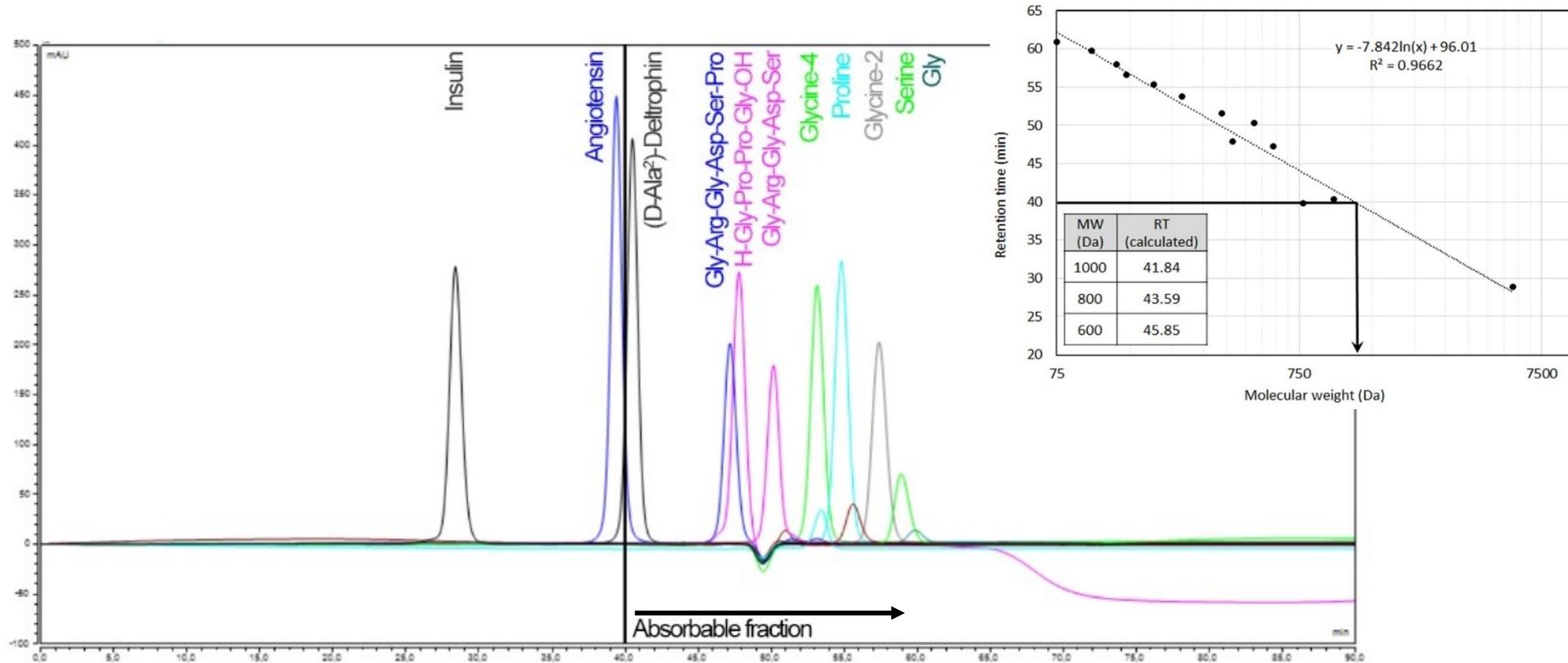


Determination of *in vitro* digestibility





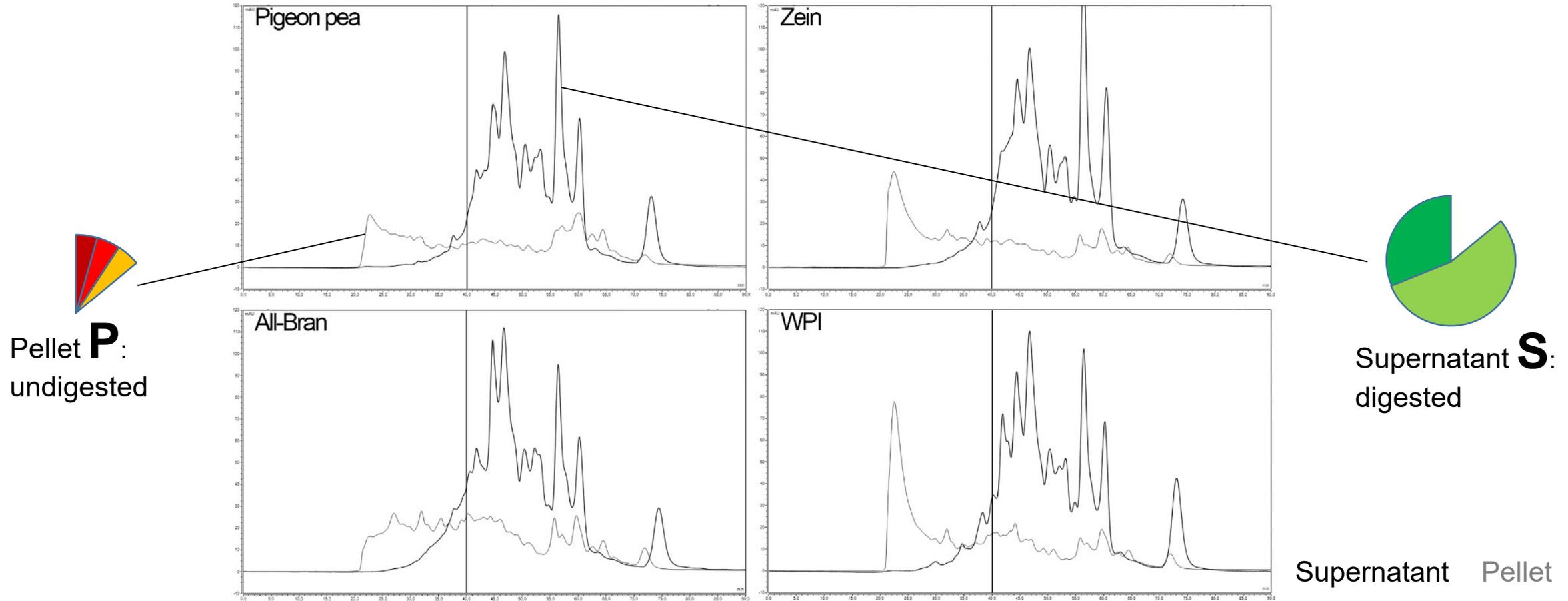
Size exclusion chromatography (SEC)



- SEC shows size distribution in samples
- Cut-off set up at 40min ~ 1000 Da



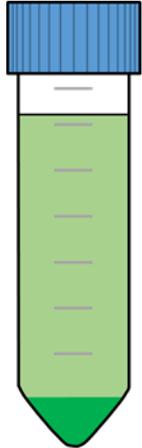
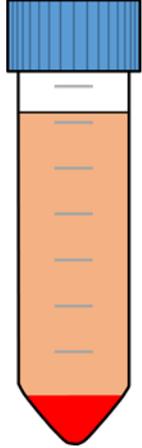
Precipitation with 80 % MeOH



→ Precipitation separates absorbable from non-absorbable components



The different analytical endpoints

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



Digestible indispensable amino acid score (DIAAS)

mg amino acid per g food protein

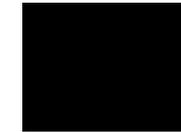


mg indispensable amino acid per g food protein



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.

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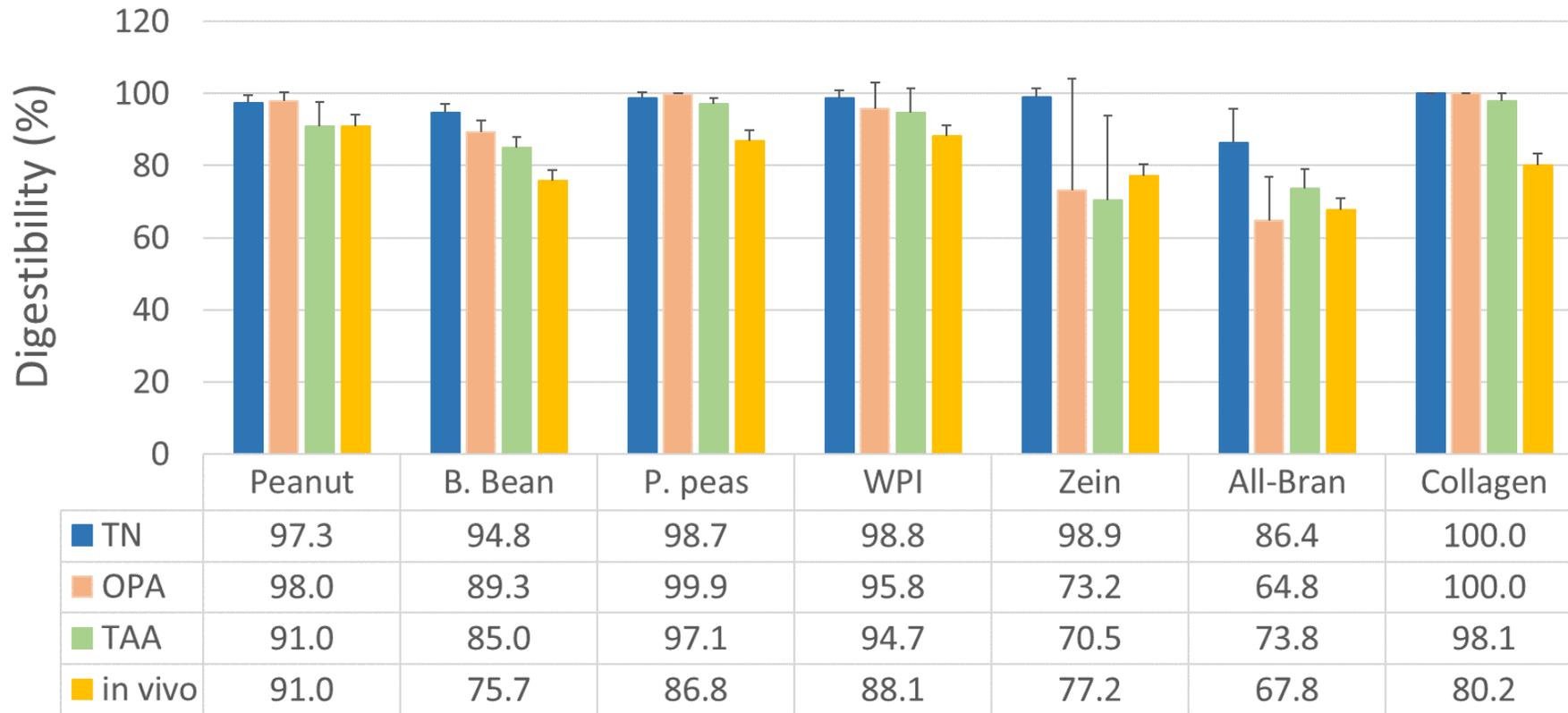
DIAAS: Digestible indispensable amino acid score

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

*lowest DIAAS is reported as limiting amino acid



Proteos *in vitro* versus *in vivo* digestibility



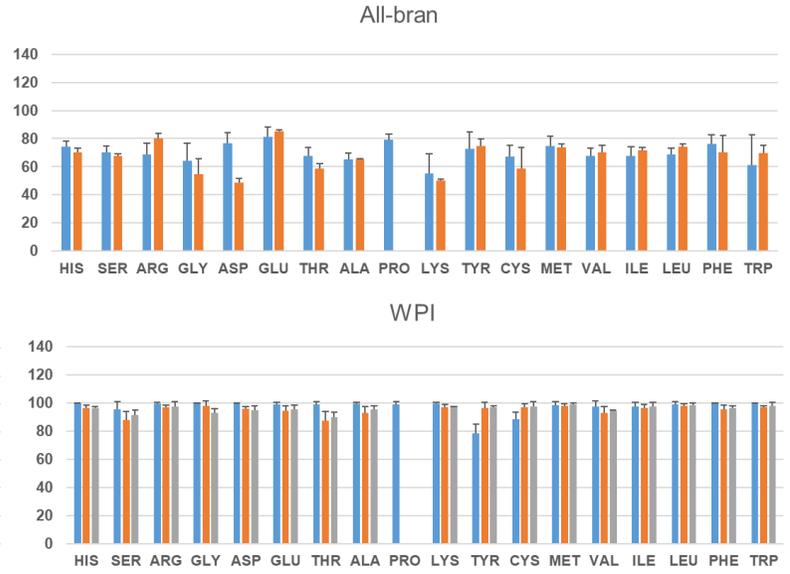
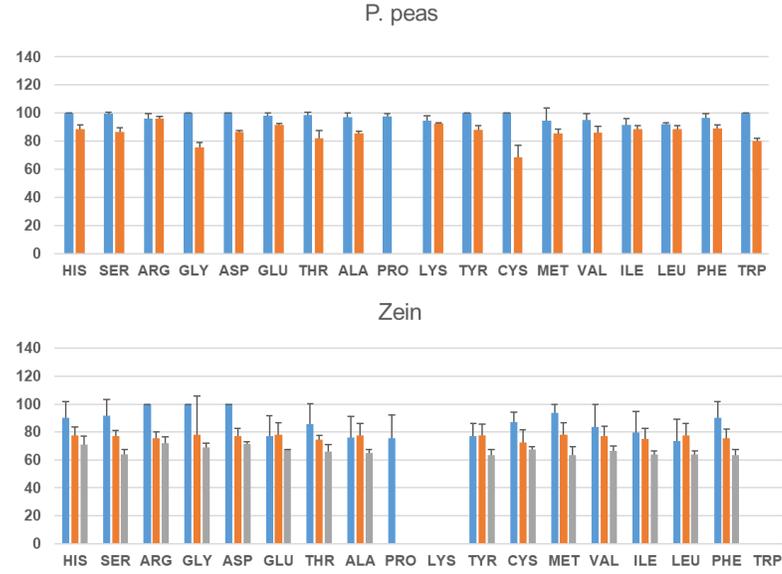
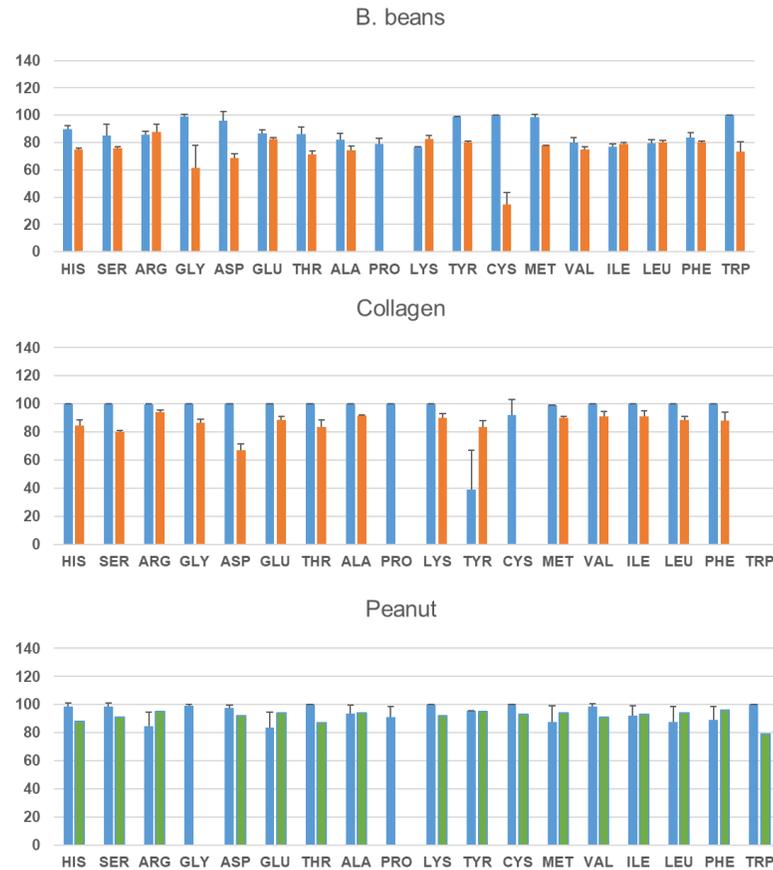
→ In general the three methods give similar results

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



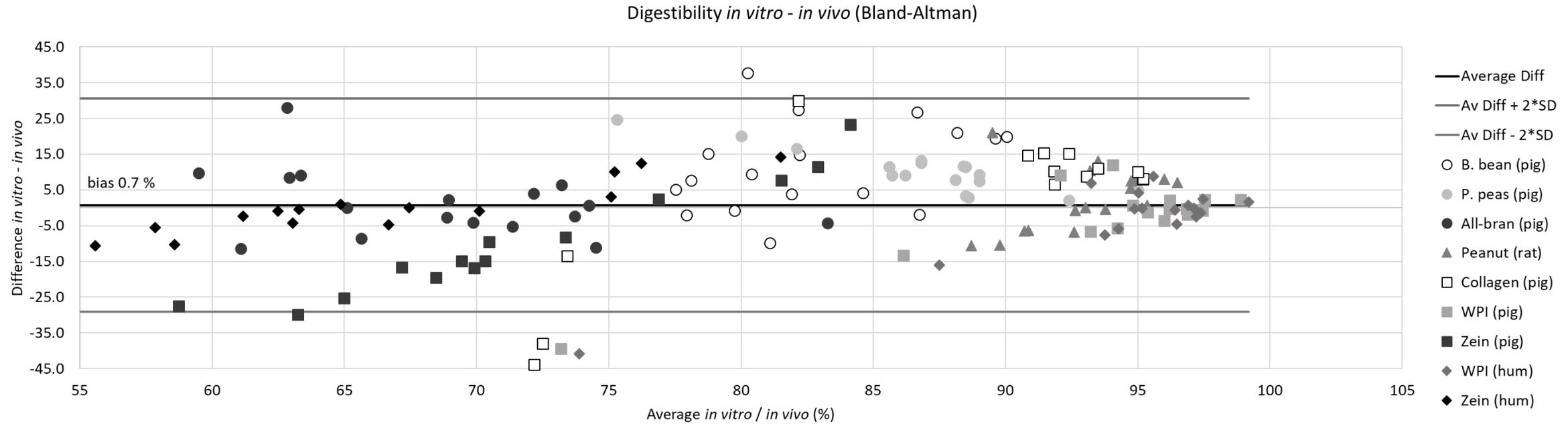
Individual amino acids digestibility

Digestibility (%)





Proteos *in vitro* versus *in vivo* digestibility all substrates



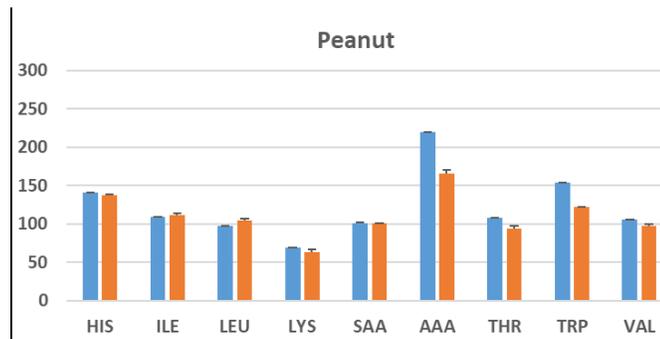
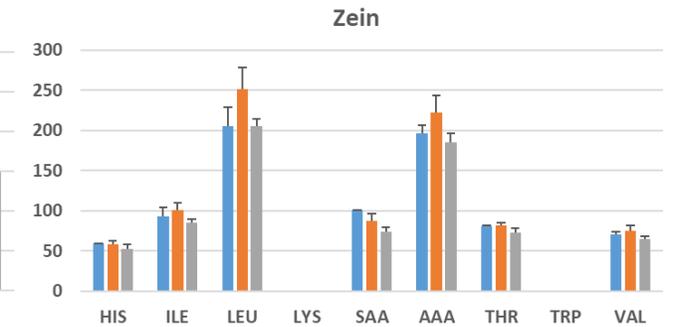
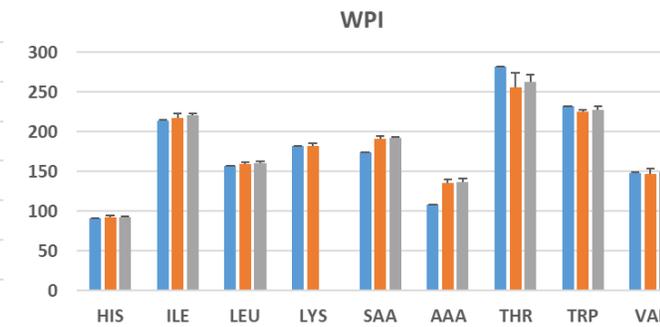
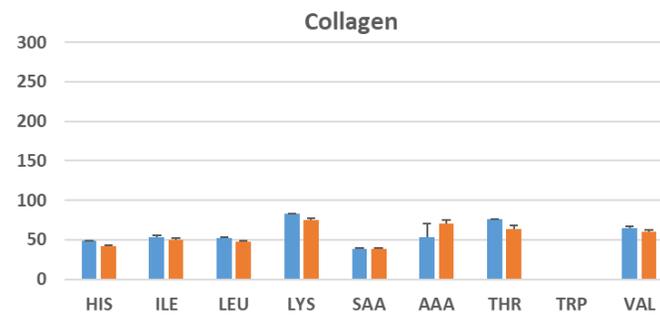
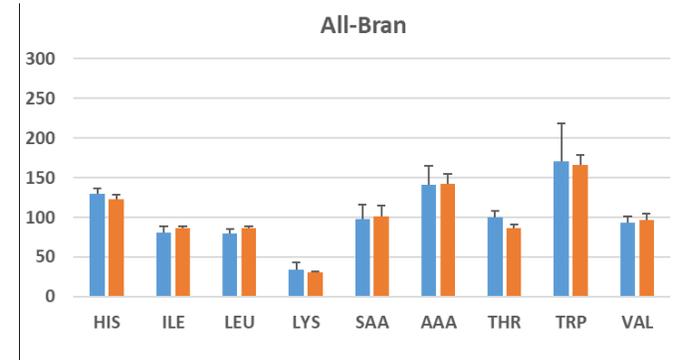
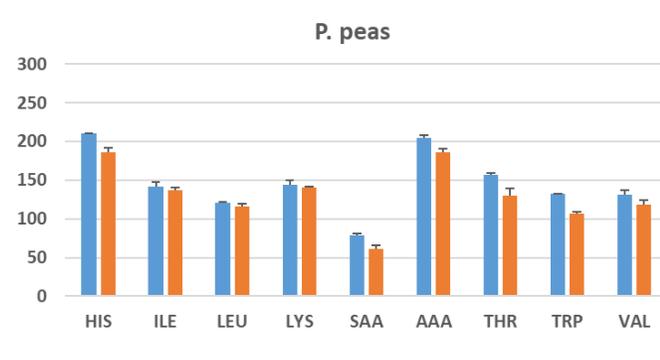
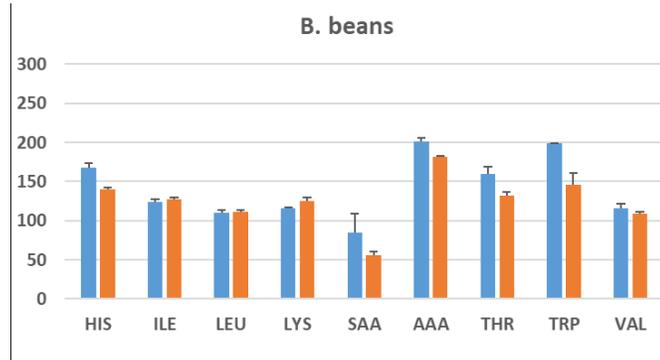
→ The two methods are in agreement

→ bias: 0.7 %



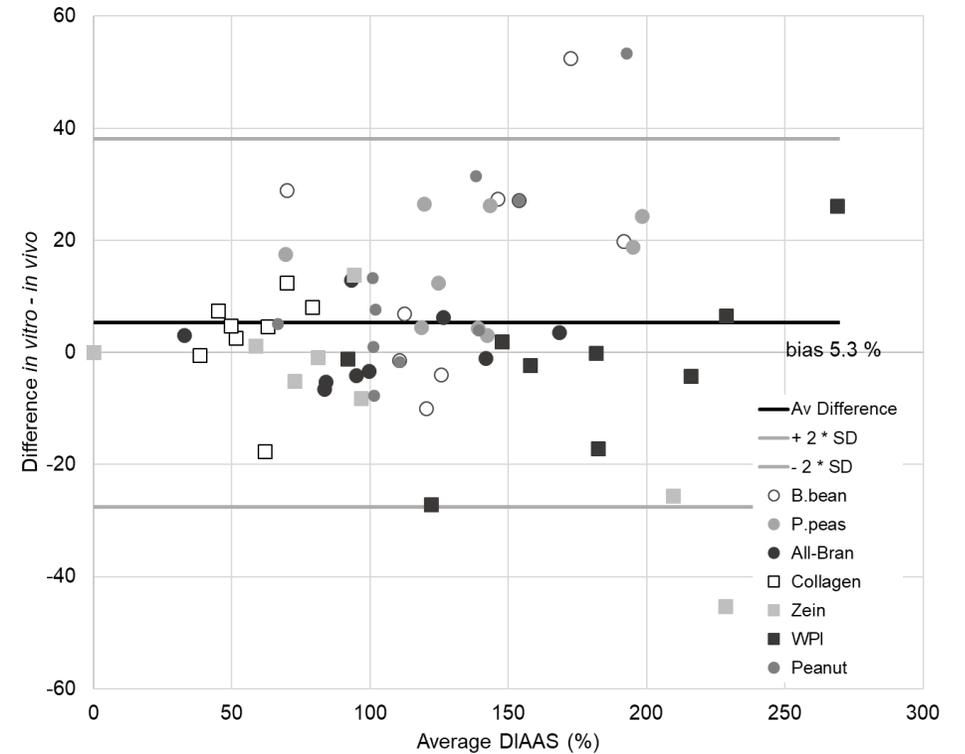
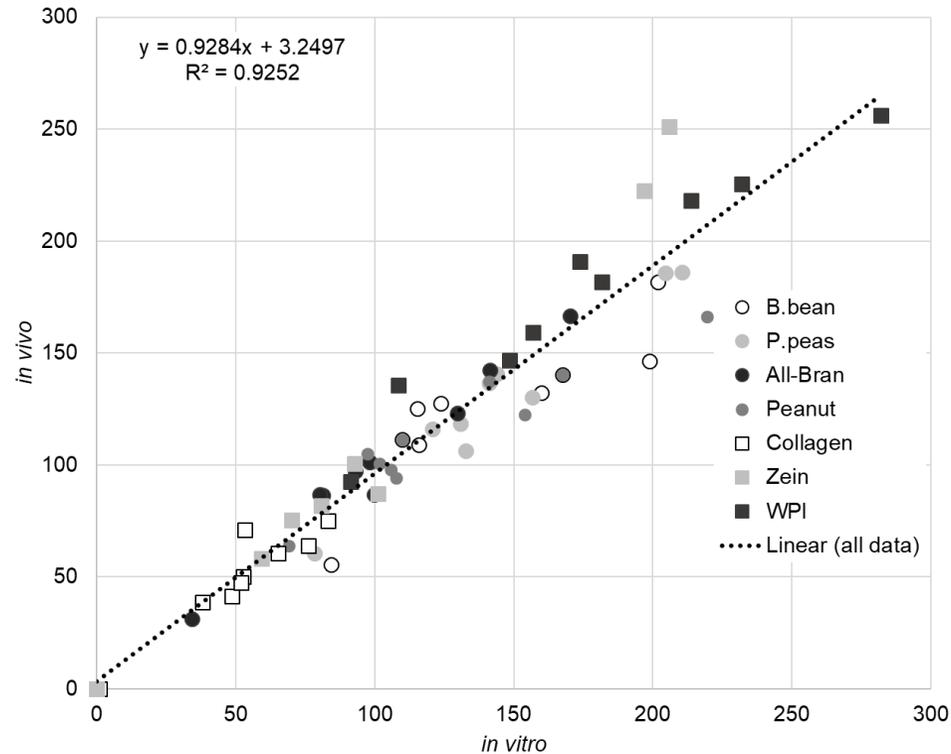
Proteos *in vitro* versus *in vivo* DIAAS

DIAAS (%)





Proteos *in vitro* versus *in vivo* DIAAS



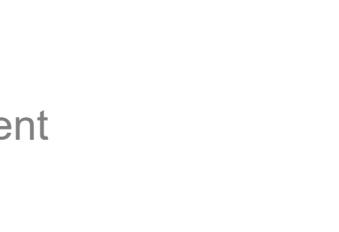
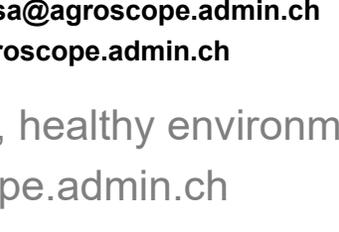
→ Good correlation *in vivo* DIAAS versus *in vitro* DIAAS

→ bias: 5.3 %



Conclusions

- These *in vitro* conditions provide a good estimate of the *in vivo* situation for total digestibility, digestibility of individual amino acids and DIAAS
- The adapted INFOGEST *in vitro* digestion protocol is suitable for measuring digestibility of food
- This protocol was only tested with seven different protein sources so far. In the future the method has to be validated with more protein sources



Thank you for your attention

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