In vitro digestibility of plant-based protein foods and ingredients using the INFOGEST workflow *vs.* standardized values in growing pigs

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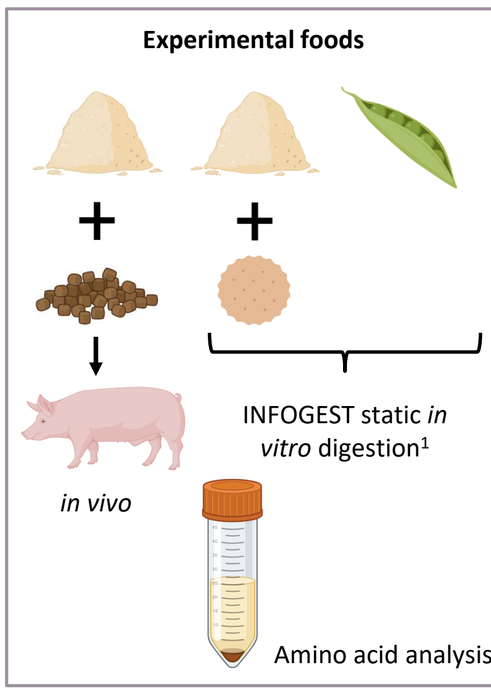
INTRODUCTION

- It exists an increasing interest in developing *in vitro* methods to evaluate protein nutritional quality, avoiding animal models or human trials.
- These *in vitro* models should allow the prediction of true ileal protein digestibility at the level of individual amino acids and the calculation of *in vitro* digestible indispensable amino acid scores (DIAAS). However, there is a lack of comparative data between *in vitro* and *in vivo* generated DIAAS values

OBJECTIVES

- Determine the *in vitro* digestibility of amino acids in ten plant-based protein ingredients
- Compare with the true (standardized) ileal digestibility in cannulated growing pigs
- Milk products were used for reference as highly digestible animal-derived protein sources

MATERIALS AND METHODS



Composition of experimental diets (*in vivo*)		
Item (%)	Test ingredient	Nitrogen-free diet
Protein	13.5-16	-
Corn starch	53-55	69.25
Soybean oil	4	4
Sucrose	20	20
Others (salts, bulking agents, vitamins)	6.5-6.7	6.75

Composition of test ingredient and nitrogen-free cookie (*in vitro*) *		
Item (%)	Test ingredient	Nitrogen-free cookie
Protein	40	-
Corn starch	10.2	40.8
Margarine	9.23	36.9
Sucrose	3.93	15.7
Cellulose	1.23	4.9
Others (Baking powder and ground ginger)	0.3	1.2

* Whole food test ingredients were digested without food matrix for *in vitro* assays

Amino acid analysis was performed by UPLC. Sulphur amino acids and Tryptophan are still in progress

RESULTS

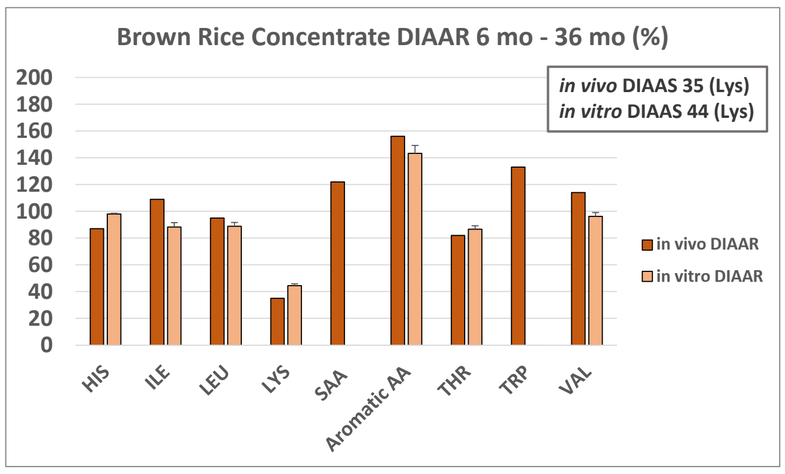
Table 1: *In vivo* standardized ileal digestibility of crude protein (%) and comparison with *in vitro* digestibility by two diferent methods: free amino groups (OPA dig) and amino acid analysis (AA dig).

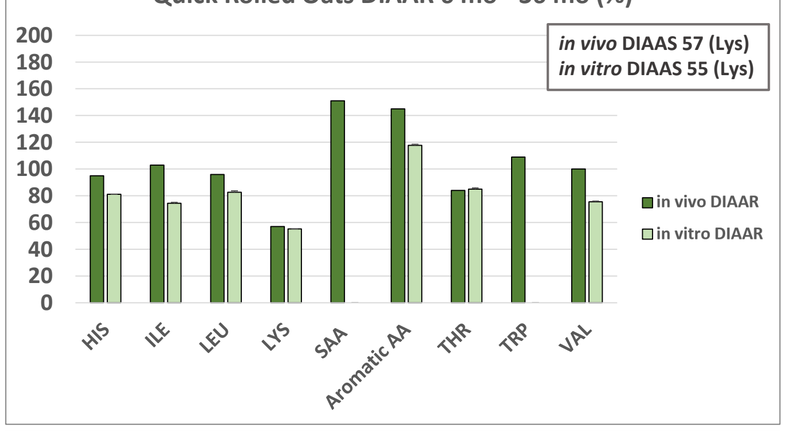
Substrate	*IN VIVO*	*IN VITRO*		*in vitro AA vs in vivo* (%)
	CP dig	OPA dig	AA dig	
Soy protein isolate + PFC	97.2[2]	95.4 ± 4.9	94.6 ± 1.5	2.7
Brown rice concentrate + PFC	75.1[2]	68.5 ± 2.8	75.1 ± 4.4	0.0
Pea protein concentrate + PFC	94.8[2]	89.8 ± 1.2	94.1 ± 0.9	0.7
Pea protein isolate + PFC	93.6[3]	94.3 ± 4.9	93.7 ± 2.4	0.1
Rapeseed protein isolate + PFC	78.9[2]	95.2 ± 4.4	93.1 ± 4.7	18.0
Rapeseed protein isolate heat treated + PFC	96.5[2]	92.9 ± 0.6	93.7 ± 2.7	2.9
Whey protein isolate + PFC	95.0[4]	90.1 ± 0.8	89.8 ± 1.7	5.4
Corn flakes	98.6[5]	73.9 ± 5.4	74.5 ± 3.5	24.5
Quick rolled oats	91.9[5]	95.4 ± 6.6	94.2 ± 2.1	2.5
Freeze-dried cooked navy beans	96.6[6]	82.5 ± 0.6	85.6 ± 0.9	11.4
Freeze-dried boiled green beans	85.8[6]	64.8 ± 2.5	80.9 ± 2.1	5.8
Skim milk powder	95.0[7]	97.2 ± 4.8	94.9 ± 0.9	0.1

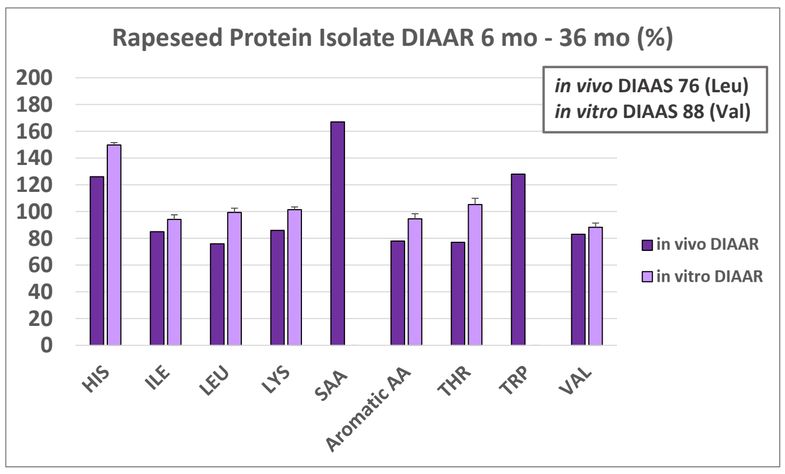
Superscript number in the *in vivo* crude protein digestibility column corresponds to the literature reference. PFC, protein free cookie; CP dig, crude protein digestibility; OPA dig, *in vitro* digestibility calculated by OPA; AA dig, *in vitro* digestibility calculated by amino acid analysis

- Comparable values of *in vivo* and *in vitro* protein digestibility except for rapeseed protein isolate, corn flakes and navy beans.
- The low *in vivo* standardized ileal digestibility in rapeseed protein isolate was attributed to the presence of trypsin inhibitor activity, but the *in vitro* method was not affected, due to the excess of trypsin in the intestinal phase.
- Lower *in vitro* protein digestibility was found for corn flakes probably due to low protein content of the test food. The effect of the food matrix composition will be investigated.

- Similar *in vivo* and *in vitro* DIAAR values for most ingredients was found, but rapeseed protein isolate showed higher in vitro DIAAR.
- Heat treatment of the rapeseed protein isolate decreased the trypsin inhibitor activity and comparable *in vivo/in vitro* DIAAR values were obtained. However the different matrix used might affect particular amino acid digestibilities, and in turn, the DIAAS.







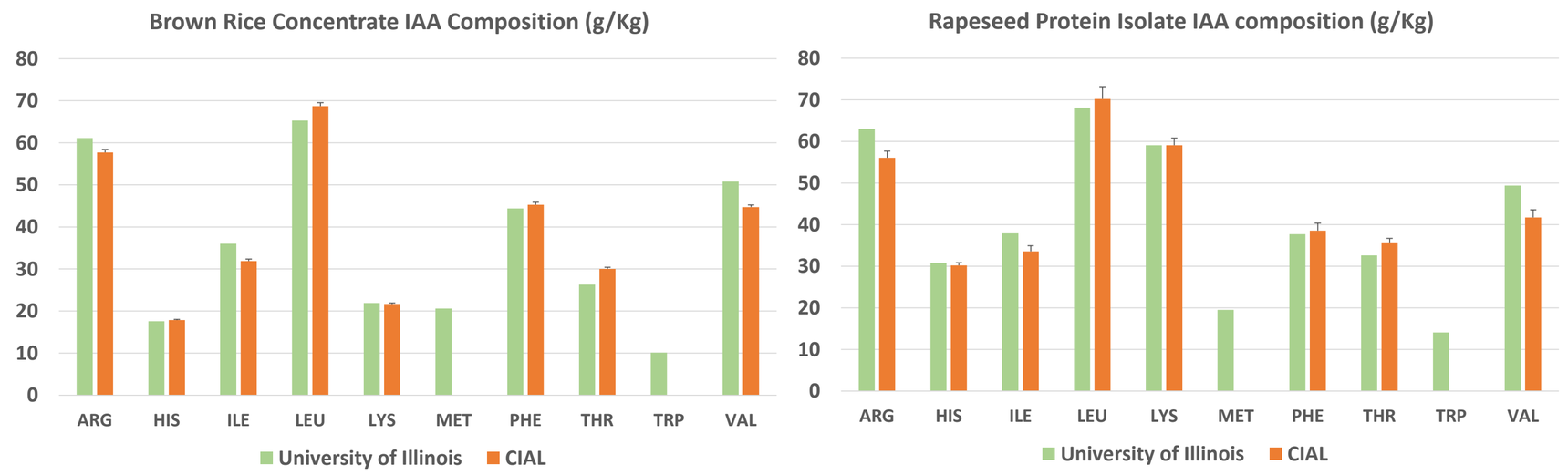


Fig. 1: Indispensable amino acid composition comparison of two products at University of Illinois and CIAL

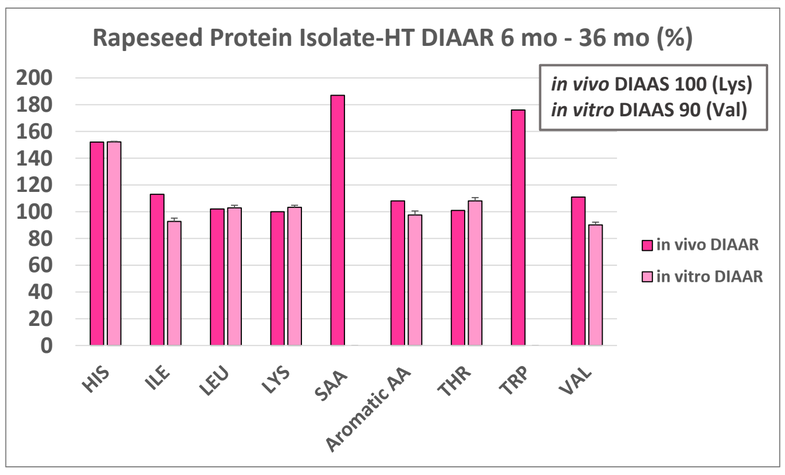


Fig. 2: Standardized ileal *vs in vitro* amino acid digestibility of brown rice concentrate (similar protein digestibility) and rapeseed protein isolate where *in vitro* digestibility was overestimated

Fig. 3: *In vivo vs in vitro* digestible indispensable amino acid ratio (DIAAR) for three protein concentrates and quick rolled oats

CONCLUSIONS

- Comparable protein digestibility *in vitro vs in vivo* except for rapeseed protein isolate, corn flakes and navy beans.
- *In vitro* digestion does not seem to be affected by trypsin inhibitory activity of rapeseed protein isolate.
- The composition of the experimental diet could affect protein digestibility. *In vitro* digestions using the same composition of the pig experimental diets are in progress.

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