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Objectives: The measurement of amino acid (AA) digestibility of protein through direct ileal sampling is highly invasive and inappropriate for vulnerable populations, such as children or elderly. The new dual tracer method relies on comparing meal and plasma isotopic ratios of 1/a test protein 2/a reference protein (or AA mix) of known digestibility, each one being labelled with a different isotope. The aim of this study was to compare this new indirect dual tracer method to standard ileal method, for the determination of AA digestibility of pea protein and milk casein.

Methods: Fifteen healthy adult volunteers completed the study and were equipped with a naso-ileal tube. They were given 9 portions of mashed potatoes containing either pea protein or casein isolates that were intrinsically labelled with ^{15}N and ^{2}H. A ^{13}C algal free AA mix was added in the meals as the reference for dual tracer method. Plasma samples were collected regularly from before the first ingestion to 8-h later, while ileal digesta were collected continuously. For ileal sampling method, the AA digestibility (RID_{AA}) was determined using the recovery a non-absorbable marker (PEG-4000) perfused in the ileum, and the measurement of ^{15}N enrichment of the digesta. For the dual tracer method, the amount of AA absorbed (Ab_{AA}) was calculated by the ratio of $^{2}H/^{13}C$ enrichments in plasma and in meals. The isotopic enrichments were evaluated in digesta, plasma samples and meals by GC-C-IRMS. The AA content was measured in digesta and meals by U-HPLC.

Results: Mean Ab_{AA} and RID_{AA} of pea protein were 102.2 $\pm$ 3.1% and 94.3 $\pm$ 1.5%, respectively. Mean Ab_{AA} and RID_{AA} of casein were 91.9 $\pm$ 2.0% and 97.1 $\pm$ 0.8%, respectively. The dual tracer method overestimated by 10% and 5% the AA digestibility of pea protein and casein, respectively, and the variability was high. The mean ileal AA digestibility of the ^{13}C free AA mix was high (98.1 $\pm$ 1.1%), which validated our choice to use it as the reference 'protein' in the dual tracer method.

Conclusions: Several AA digestibilities obtained with dual tracer method were in the same range as the digestibilities from ileal sampling method. The variability was high and the effect of the protein source was inconsistent. After further research and validation, the dual tracer method could lead to notable advances in the determination of protein quality in humans.

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