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Confrontation of the “Dual Tracer” Indirect Method With Direct Ileal Sampling for Indispensable Amino Acid Digestibility of Sunflower Isolate in Humans

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Objectives: The direct assessment of ileal samples of amino acid (AA) digestibility is invasive in humans. A less invasive but indirect method, namely « dual tracer » was recently developed. It relies on the plasma isotopic enrichment ratio of two labeled protein, a reference protein or alternately AAs labeled with ¹³C and the test protein labeled with ¹⁵N. This recent method has not yet been challenged against direct measurement of ileal digestibility.

Methods: Seven healthy volunteers were intubated with naso-ileal tube. Every 30min for 4h, they ingested sunflower biscuits containing a total of 25g of ¹⁵N intrinsically labeled sunflower protein isolate. They also ingested 60g chocolate containing a total of 400mg of a mix of ¹³C algal individual AAs. Ileal contents were collected continuously for 8h following the first meal and plasma was sampled every 30min for 4h and hourly between 4 and 8h. ¹⁵N and ¹³C indispensable amino

acid (IAA) ileal digestibility were determined by measuring ¹⁵N and ¹³C enrichment in AAs by GC-C-IRMS and AA content by UHPLC in ileal effluent. Plasma and meal ¹⁵N and ¹³C IAA enrichment were measured by GC-C-IRMS. Isotopic ¹⁵N/¹³C ratio were determined using area under the curve value for each isotope.

Results: Using direct ileal sampling, average IAA ileal digestibility was: (i) $88.5 \pm 5.0\%$ for sunflower isolate (¹⁵N) with values ranged from $85.8 \pm 5.1\%$ for threonine to $91.1 \pm 5.8\%$ for methionine, and (ii) $97.6 \pm 1.7\%$ for free AAs (¹³C) with values ranged from $95.9 \pm 2.3\%$ for lysine to $98.8 \pm 0.8\%$ for phenylalanine. With the “dual tracer” method, digestibility of isoleucine, leucine, threonine and valine was significantly lower than with ileal determination (from 7.9% for threonine to 24.3% for leucine), Methionine and phenylalanine values were aberrant (over 100%) For lysine, the difference between the two methods was not statistically different (4.7%, $p = 0.49$).

Conclusions: With our methodological conditions, the “dual tracer” method provides physiological values for most IAA except methionine and phenylalanine. However, values were low compared to ileal digestibility (about 10%) and interindividual variability was high. This less invasive method is promising but requires methodological improvements.

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