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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 <sup>13</sup>C and the test protein labeled with <sup>15</sup>N. 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 <sup>15</sup>N intrinsically labeled sunflower protein isolate. They also ingested 60g chocolate containing a total of 400mg of a mix of <sup>13</sup>C 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. <sup>15</sup>N and <sup>13</sup>C indispensable amino

acid (IAA) ileal digestibility were determined by measuring <sup>15</sup>N and <sup>13</sup>C enrichment in AAs by GC-C-IRMS and AA content by UHPLC in ileal effluent. Plasma and meal <sup>15</sup>N and <sup>13</sup>C IAA enrichment were measured by GC-C-IRMS. Isotopic <sup>15</sup>N/<sup>13</sup>C 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 (<sup>15</sup>N) 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 (<sup>13</sup>C) 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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