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Challenging in Rats the Use of ^{13}C Spirulina as Reference Protein for the Dual Isotope Method to Determine Amino Acid Bioavailability (P08-061-19)

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Objectives: In order to establish DIAAS in humans, the FAO recommended to develop a new method to measure indispensable amino acid (IAA) digestibility. This method uses two isotopic labeling, one for the protein to test and one for a reference protein. Spirulina was chosen as the ^{13}C reference protein due to its commercial availability and affordability. However, the real digestibility of spirulina protein has not been measured *in vivo*. This work aims to assess the digestibility of spirulina and its repeatability in different meal tests in rats.

Methods: 23 Wistar male rats were fed a test meal containing 0.5 g of ^{15}N protein from either spirulina ($n = 7$), sunflower $n = 8$ or goat milk isolate ($n = 8$) and 10 mg of ^{13}C labeled spirulina. Rats were euthanized 6 h after the meal and their digestive luminal contents (stomach, small intestine, ileum, caecum, colon) were collected. Protein digestibility was determined for the test and the reference proteins by measuring ^{15}N and ^{13}C enrichments in the digesta by EA-IRMS. Caecal IAA digestibility of ^{13}C spirulina was determined by measuring the quantity of AA in the

caecum by UPLC and the ^{13}C enrichment in AA by GC-C-IRMS. Group effects were tested using one way ANOVA and differences between groups using Bonferroni test.

Results: Six hours after ingestion, most of the dietary ^{15}N and ^{13}C were found in the caecum and colon. But there at least twice more ^{15}N nitrogen in the caecum and colon in the spirulina group than in the two other groups. Therefore, spirulina protein digestibility ($86.0 \pm 0.7\%$) was lower ($P < 0.001$) than sunflower ($95.1 \pm 0.5\%$) and goat milk digestibility ($97.2 \pm 0.2\%$). ^{13}C spirulina digestibility tended to be different ($P = 0.06$) when mixed to spirulina ($90.6 \pm 0.6\%$), sunflower ($88.8 \pm 0.5\%$) or goat milk ($89.0 \pm 0.5\%$) isolates. The caecal IAA digestibility of ^{13}C spirulina was lower in the spirulina group than in sunflower and goat milk groups for every IAA tested, and the mean was $91.6 \pm 0.2\%$ for sunflower, $91.4 \pm 0.4\%$ for goat milk and $85.4 \pm 0.6\%$ for spirulina.

Conclusions: Spirulina protein is of lower digestibility than other animal or plant proteins. Protein and amino digestibility of a tracer dose of ^{13}C spirulina appears to vary depending on the protein component of the meal. These results question the use of spirulina as a reference protein for the dual isotope method.

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Supporting Tables, Images and/or Graphs

	Val	Leu	Ile	Thr	Phe	Val	Met	IAA Mean
Sunflower	91.5 ± 0.4 a	91.5 ± 0.4 a	91.0 ± 0.3 a	91.8 ± 0.4 a	89.6 ± 0.5 a	91.7 ± 0.4 a	93.4 ± 0.3 a	91.6 ± 0.2 a
Goat milk	91.0 ± 0.8 a	91.0 ± 0.9 a	91.4 ± 0.8 a	91.7 ± 0.9 a	88.7 ± 1.1 a	92.4 ± 0.8 a	93.3 ± 0.7 a	91.4 ± 0.4 a
Spirulina	84.8 ± 1.5 b	83.7 ± 1.6 b	84.3 ± 1.5 b	88.4 ± 1.1 b	80.4 ± 1.0 b	88.1 ± 1.1 b	88.9 ± 1.3 b	85.4 ± 0.6 b
Group effect	<0.001	<0.001	<0.001	0.01	<0.001	<0.01	<0.001	<0.001