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1 **Protein and amino acid digestibility of <sup>15</sup>N spirulina in rats**

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11

12 **Abbreviations:** AA: amino acid; Asx: asparatate and asparagine; APE: atom percent excess;  
13 CS: Chemical Score; DM: dry matter; EA: elemental analyzer; GC-C: gas chromatography  
14 coupled to a combustion furnace; Glx: glutamate and glutamine; IAA: indispensable amino  
15 acid; IRMS: isotope-ratio mass spectrometer; N: nitrogen; SEM: standard error of the mean;  
16 PD-CAAS: protein digestibility- corrected amino acid score SS: sonicated spirulina; UHPLC:  
17 ultra-high-performance liquid chromatography

18 **Abstract**

19 Purpose:

20 Spirulina is often used as dietary supplement for its protein content and quality. However, *in*  
21 *vivo* data on protein digestibility are lacking. This study aims to determine nitrogen and amino  
22 acid digestibility in rats. A secondary objective was to test the effect of sonication prior to  
23 ingestion in order to break cell walls.

24 Methods:

25 Wistar rats were fed a single test meal containing  $^{15}\text{N}$  spirulina that was either sonicated (n=11)  
26 or not (control, n=13). Rats were euthanized 6 h after the meal ingestion. Spirulina nitrogen  
27 digestibility was measured by assessment of  $^{15}\text{N}$  recovery in digestive contents. Amino acid  
28 digestibility was measured by quantification of the caecal amino acid content and their  $^{15}\text{N}$   
29 enrichment.

30 Results:

31 Real fecal nitrogen digestibility was  $86.0\pm 0.7\%$ , without any differences between groups. Mean  
32  $^{15}\text{N}$  amino acid caecal digestibility was  $82.8\pm 1.3\%$ , and values ranged from  $77.9\pm 1.9\%$  for  
33 serine to  $89.4\pm 1.0\%$  for methionine. No effect of sonication was observed. The most limiting  
34 AA was histidine, with a chemical score of 0.98 and a PD-CAAS of 0.84. Lysine was also  
35 limiting in a lesser extent.

36 Conclusion:

37 The nitrogen and amino acid digestibility of spirulina is relatively low, and showed no effect of  
38 prior sonication. Its amino acid composition is relatively well balanced but not enough to  
39 compensate for the poor digestibility.

## 40 **Introduction**

41 Spirulina is a cyanobacteria of the genus *Arthrospira*. Two species, *A. platensis* and *A.*  
42 *maxima*, are primarily used in food supplements for their high protein content, which varies  
43 between 50 and 70%, depending on growing conditions [1]. They also contain large amounts  
44 of  $\beta$ -carotene, vitamin B12 and iron [1], which are presumed to have health benefits such as  
45 anticancer, antibacterial and antiviral effects [1]. Furthermore, it has been shown that the  
46 protein amino acid composition of spirulina meets FAO requirements [2]. Although spirulina  
47 contains less histidine and methionine than bovine milk [3], its composition is more balanced  
48 than many plant protein sources, making it an potentially useful amino acid source.

49 However, there are few studies on spirulina protein and amino acid bioavailability. In a human  
50 study that implemented the dual isotope method, a novel, non-invasive method was used to  
51 determine amino acid digestibility in humans by comparing plasma appearance of amino acids  
52 to that of free amino acids. The authors found that spirulina amino acid digestibility ranged  
53 from 77.5% for lysine to 95.3% for phenylalanine with a mean indispensable amino acid  
54 digestibility of 85.2% [4]. This study showed a relatively low protein digestibility that could be  
55 explained by the presence of cell walls. In a study on *Chlorella vulgaris*, another microalgae, it  
56 was demonstrated that ultrasonication tended to improve true fecal protein digestibility by 10%  
57 in rats [5]. One possible explanation is that sonication, by application of sonic energy, could  
58 enhance protein digestibility of spirulina by breaking cell walls.

59 This study aimed to evaluate the real digestibility of spirulina nitrogen and amino acids *in vivo*  
60 in rats using  $^{15}\text{N}$  labeled spirulina and to assess the effect of sonication.

## 61 **Materials and methods**

### 62 **Animal and diets**

63 This experiment was executed in compliance with ARRIVE guidelines, the EU directive  
64 2010/63/EU for animal experiments, and were approved by Ethics committee in animal  
65 experiment of INRA (N°17-20 and 16-44, Jouy-en-Josas, France) and the French Ministry of  
66 Higher Education and Research (APAFIS#11921-2017091818236657 and APAFIS#9769-  
67 2016102617397786). Male Wistar rats (Envigo, Huntingdon, United Kingdom) (n=24),  
68 weighing 250-275 g at their arrival were housed individually, in cages with grids to avoid  
69 coprophagy, in a reversed 12h light/dark cycle and in standard conditions (free access to tap  
70 water and food). They were fed a standard diet (Table 1). After acclimation, rats were sorted  
71 into two groups according to the spirulina meal they would receive: control spirulina (CS, n=13)  
72 or sonicated spirulina (SS, n=11). From day 4 to 13, rats followed an alimentary protocol in  
73 order to accustom them to consume a single meal in the morning. The protocol consisted of  
74 two meals: a morning meal of 4 g of standard diet for 30 mins (from 8:30 to 9:00 a.m.) and an  
75 *ad libitum* meal for 6 hours (from 12:00 to 6:00 p.m.). Between these two meals, rats were  
76 fasted with free access to tap water (Fig 1). On day 8, in order to prevent neophobia, rats  
77 received a 4 g meal containing spirulina during the dark phase. On day 13, they were fed a  
78 complete 4 g meal containing 0.77 g of spirulina (Cambridge Isotope Laboratories, Andover,  
79 USA), either sonicated or not. The meal was extrinsically labeled by adding 10 mg of <sup>15</sup>N  
80 spirulina (98%, Cambridge Isotope Laboratories, Andover, USA), sonicated or not. Unlabeled  
81 and labeled spirulina were sonicated for 1 h 30 min at 35 kHz (Elmasonic One, Elma  
82 Schmidbauer GmbH, Singen, Germany) and then freeze dried. The spirulina composition,  
83 provided by the supplier, was composed of 60.9% protein (Kjeldahl 6.25), 6.5% fat, 4.2% fiber  
84 and 10.2% ash.

85 The test meal composition was similar to the standard diet (Table 1). The spirulina amino acid  
86 composition is given in Table 2. As previously described [6–8], six hours after the meal, rats  
87 were intraperitoneally given a lethal dose of pentobarbital sodium (100 mg/mg) and

88 exsanguinated. Gastro-intestinal segments were identified as stomach, proximal small  
89 intestine, ileum (defined as the last 10 cm before the caecum), caecum and colon. Luminal  
90 contents of these segments were rinsed with NaCl solution (9‰), collected entirely, weighed  
91 and stored at -20°C. Feces were collected during the 6 h following the test meal and were  
92 added to colonic contents.

93

#### 94 **Analytical methods**

95 Nitrogen digestibility was assessed by measuring <sup>15</sup>N recovery in digestive contents. Nitrogen  
96 percentage and <sup>15</sup>N enrichments in digestive samples and meal were determined by an  
97 elemental analyzer (Vario Micro Cube, Elementar, Lyon, France) coupled with isotopic ratio  
98 mass spectrometry (IRMS) (Isoprime, GV Instrument, Manchester, UK). Atropine (Thermo  
99 Electron, Milano, Italy) and L-glutamic acid (USGS41, Sigma Aldrich, Saint-Louis, USA) were  
100 used as elemental and isotopic standards, respectively.

101 Proteins were hydrolyzed for 24 h with HCl 6N at 110°C. Amino acids were isolated using a  
102 hydrogen form ion exchange resin (Dowex® 50WX8 hydrogen form 100-200 mesh, Sigma-  
103 Aldrich, Saint-Louis, USA) and derivatized with ethyl chloroformate. <sup>15</sup>N enrichments in meal  
104 and digestive luminal contents were analyzed by gas chromatography (GC 6890N, Agilent  
105 Technologies, Les Ulis, France) coupled to an Isoprime isotope ratio mass spectrometer  
106 (Isoprime, GV Instrument, Manchester, UK) *via* the GC5 Isoprime interface (350°C). The  
107 furnace temperature was 950°C. The inlet temperature was set at 270°C. Samples (2µl) were  
108 injected in splitless mode for <sup>15</sup>N analysis. The GC column temperature program started at  
109 150°C, rose to 200°C at 4°C/min and then to 270°C at 25°C/min. The temperature was  
110 maintained for 20 min at 270°C.

111 To quantify tryptophan in spirulina, 15 mg of spirulina were hydrolyzed for 20h at 110°C in the  
112 presence of barium hydroxide 2N. Norvaline and 5-methyl-tryptophan were used as internal  
113 standards and were added before alkaline hydrolysis. To quantify amino acids other than

114 tryptophan, 10 mg of digestive contents or meal were hydrolyzed for 24 h with HCl 6N at 110°C.  
115 For sulfur amino acids quantification in the meal, performic acid oxidation was used before  
116 acid hydrolysis to convert methionine and cysteine to methionine sulfone and cysteic acid.  
117 Norvaline was used as an internal standard, added before acid hydrolysis. Calibration  
118 standards were composed of a mixture of amino acids (Waters, Milford, USA) to which  
119 norvaline, methyl-tryptophan, cysteic acid and methionine sulfone were added depending on  
120 amino acids quantification. Amino acids and standards were derivatized using the AccQ-Tag  
121 Ultra Derivatization kit (Waters, Milford, USA) according to the manufacturer's protocol. Amino  
122 acid analysis was performed on an Acquity H-class UHPLC system with a PDA detector  
123 (Waters Corporation, Milford, USA). Amino acids were separated using AccQ-Tag amino acid  
124 C18 column (2.1 × 100 mm; 1.7 µm bead size; Waters Corporation, Milford, USA). Column  
125 temperature was 43°C and the eluents were: AccQ-Tag Ultra Eluent A (Waters Corporation,  
126 Milford, USA), 2% formic acid in acetonitrile, and MilliQ water. The solvent gradients were run  
127 at 0.7 ml/min and injection volume was 1 µl.

## 128 **Calculations**

129 Exogenous (i.e. dietary) nitrogen ( $N_{\text{exo}}$  in mmol) in gastro-intestinal contents was calculated as  
130 follows:

$$131 \quad N_{\text{exo}} = N_{\text{tot}} \times \frac{APE_s}{APE_m}$$

132 Where  $N_{\text{tot}}$  is the amount of nitrogen (N) in the sample and APE the enrichment excess  
133 compared to the natural enrichment (in atom percent) of  $^{15}\text{N}$  in the sample or the meal (s or  
134 m).

135 As amino acids and peptides are absorbed primarily in the duodenum and jejunum, undigested  
136 food nitrogen were determined from N recovery in distal parts of the intestine (ileum and large  
137 intestine, including feces). As digestion was almost complete after 6 h, losses due to residual  
138 N in the stomach and intestine were considered negligible. Accordingly, fecal nitrogen  
139 digestibility (in % of N) was determined as follows:

140 
$$\text{Fecal nitrogen digestibility}_N = \frac{N_{\text{ing}} - (N_{\text{exo ileum}} + N_{\text{exo caecum}} + N_{\text{exo fecal}})}{N_{\text{ing}}} \times 100$$

141 Where  $N_{\text{ing}}$  (mmol) is the amount of N ingested by the rats. Caecal nitrogen digestibility was  
 142 also assessed with this same formula, without the fecal N amount.

143 Caecal amino acid digestibility was calculated using  $^{15}\text{N}$  amino acid enrichments in the caecum  
 144 by GC-C-IRMS and amino acid quantification by UHPLC:

145 
$$\text{Caecal amino acid digestibility}_N = \left( 1 - \frac{\text{caecal AA}_i \text{ mass} \times \frac{^{15}\text{N AA}_i \text{ caecum}}{^{15}\text{N AA}_i \text{ meal}}}{\text{ingested AA}_i \text{ mass}} \right) \times 100$$

146 Mean amino acid digestibility was weighted by the amino acid composition of spirulina.

147 The Chemical Score (CS) was calculated as the lowest CS ratio:

148 
$$\text{CS ratio} = \frac{\text{mg of IAA in 1 g of dietary protein}}{\text{mg of the same IAA in 1g of the reference protein}}$$

149 The Protein Digestibility Amino Acid Score (PD-CAAS) was calculated as the lowest PD-CASS  
 150 ratio:

151  $\text{PD-CAAS ratio} = \text{CS ratio} \times \text{fecal nitrogen digestibility}$ .

152 The reference protein profile was the recommended amino acid scoring pattern for older  
 153 children, adolescents and adults [9]. A nitrogen to protein conversion factor of 6.25 was used  
 154 to calculate the amount of protein in the ingredient.

155

## 156 **Statistical analysis**

157 Results are expressed as means  $\pm$  standard errors of the mean (SEM). Differences between  
 158 the two groups were tested by unpaired Student's t-test R 3.5.1 (R project). If  $P < 0.05$ ,  
 159 differences were considered statistically significant.

160 **Results**

161 **Nitrogen digestibility**

162 Spirulina nitrogen digestibility was assessed using  $^{15}\text{N}$  recovery in digestive contents. For both  
163 groups, after six hours of digestion, most of the dietary nitrogen was found in the caecum and  
164 colon (Table 3). The control group had more dietary nitrogen in the stomach and colon than  
165 the sonicated group, but there was no difference in nitrogen digestibility (Table 3). There was  
166 also no effect of sonication on caecal digestibility for  $^{15}\text{N}$ . Pooled spirulina nitrogen digestibility  
167 ( $n=24$ ) was  $86.0\pm 0.7\%$ .

168 **Amino acid digestibility**

169 Caecal amino acid digestibility was measured using  $^{15}\text{N}$  labeling (Table 4). In the control group,  
170 weighted average amino acid digestibility was  $82.8\pm 1.3\%$ . Values ranged from  $77.9\pm 1.9\%$  for  
171 serine to  $89.4\pm 1.0\%$  for methionine. In the sonicated group, values varied between  $77.7\pm 1.5\%$   
172 for serine to  $88.9\%$  for lysine and methionine, and the mean was  $81.6\pm 1.5\%$ . There were no  
173 significant differences between groups regarding amino acids or their averages. Pooled caecal  
174 amino acid digestibility was  $82.3\pm 1.0\%$ , on average. Compared to caecal nitrogen digestibility,  
175 caecal amino acid digestibility was 8% lower regardless of the group.

176 **Chemical Score and PD-CAAS**

177 The chemical score was determined after calculating the amount of protein in the ingredient  
178 using the factor  $\text{N} \times 6.25$ . The CS was 0.98 due to a marginal insufficiency in histidine. Due to  
179 the moderate protein digestibility, the PD-CAAS was 0.84 (Fig 2). Lysine was also marginally  
180 limited, with a ratio of 0.93.

## 181 **Discussion**

182 This study aimed to assess nitrogen and amino acid digestibility of spirulina in a rat model  
183 using <sup>15</sup>N intrinsically labeled spirulina and to evaluate the effect of sonication on spirulina  
184 digestibility. The results revealed a modest nitrogen digestibility and an even lower value for  
185 amino acids. According to the PD-CAAS, histidine and lysine were limiting.

186 When pooling all animals, fecal nitrogen digestibility of spirulina was 86.0±0.7%. This is a  
187 relatively low digestibility score compared to an animal protein like meat (97%) [6], and also  
188 compared to plant protein like cooked rolled oats (95.2%) or cooked peas (89%) [10].  
189 Nevertheless, spirulina nitrogen digestibility is comparable to, or lower than, fecal values found  
190 in rats for some cereals including rice, wheat or oats [10]. Protein digestibility is higher with  
191 protein isolates than with unpurified protein. For example, pea protein concentrate true fecal  
192 digestibility is 99%, while cooked bean digestibility is only 89% [10].

193 We found a lower caecal digestibility of amino acids (83%) than caecal digestibility of nitrogen  
194 (90%). This important discrepancy could be due to a non-protein nitrogen component that has  
195 already been described in microalgae [3]. Spirulina analysis by the supplier consistently,  
196 showed that if protein content represented 60.9% of spirulina using the Kjeldahl method, only  
197 43% was based on amino acid content. The presence of urea or ammonia, for instance, could  
198 have been absorbed in the caecum and colon thus leading to an overestimation of protein  
199 digestibility. We can therefore consider that spirulina protein digestibility would be  
200 approximately 82% rather than 86% concerning caecal amino acid digestibility, and even lower  
201 if we account for possible losses at the fecal level. However, determination of amino acid  
202 losses at the fecal level are considered too uncertain because of fermentation processes. The  
203 value obtained with amino acids at the caecal level, rather than nitrogen, could thus be used  
204 as a reference value for spirulina.

205 Our values of amino acid digestibility are consistent with those obtained in humans with the  
206 dual isotope method (85.2%) [4]. In the study by Devi et al. (2018), values ranged from 77.5%

207 for lysine to 95.3% for phenylalanine, while our values ranged from 80.8% for valine to 89.4%  
208 for methionine.

209 The low nitrogen digestibility of spirulina could be due to the use of whole-cell spirulina. Since  
210 previous studies have shown a low nitrogen digestibility of spirulina in different experimental  
211 models [11, 12], we tested if prior ultrasonication of freeze-dried spirulina could enhance  
212 protein bioavailability. It has been shown that sonication can improve protein extraction  
213 efficiency of microalgae by disrupting lignocellulose walls and reducing particle size [2]. Our  
214 results, however, did not show any significant effect of sonication on nitrogen and amino acid  
215 digestibility of spirulina. A prior study on a micro-algae, *Chorella vulgaris*, showed that while  
216 ultrasonication did not statistically improve true fecal protein digestibility in rats (even if there  
217 was an increase of 10%), it did improve net protein utilization and protein efficiency ratio, two  
218 other protein quality indexes [5]. In our study, we found a significant decrease of 1.3% in fecal  
219 nitrogen recovery with sonicated spirulina but without any significant differences in amino acid  
220 digestibility. Thus our results are concordant with the study by Janczyk et al. [5], in which  
221 digestibility was assessed at the fecal level, only.

222 The PD-CAAS of spirulina is lower than that observed for soy (0.96), but equivalent or higher  
223 than those of most plant protein sources already assessed in rats, such as rice (0.56, or beans  
224 and peas (0.62 and 0.86) [10]. It must be pointed out that the spirulina PD-CAAS in this study  
225 was obtained using a nitrogen to protein conversion factor of 6.25, which allows a direct  
226 comparison with other proteins. But it would be 0.94 (histidine) using a factor of 5.7, a value  
227 that is situated between the value reported by Teuling (5.08) [3] and that reported by Safi et al.  
228 [13] (6.27). 5.7 is also the mean value of the nitrogen-protein conversion factor for plant protein.  
229 As this conversion factor is more realistic than the default factor 6.25, this suggests that in fact,  
230 the amino acid imbalance in spirulina is marginal. Finally, as spirulina is not consumed as a  
231 major source of protein, even a moderate histidine deficiency is easily compensable by other  
232 sources of protein in the diet.

233 Our study has some limitations. The first is measurement of AA digestibility at the caecal and  
234 not the ileal level. The FAO proposed the rat as an alternate model to humans and pigs for  
235 DIAAS determination [9]. However, there is no standardized method to determine ileal  
236 digestibility in rats due to the impossibility of continuously collecting ileal effluents. We  
237 developed a method that is based on a relatively short postprandial period (5 to 6h), aiming for  
238 the best compromise between limiting the fermentation time and to achieving complete  
239 digestion of the test meal. We have used it to measure protein digestibility of various protein  
240 sources [14, 15, 6, 16] and more recently, amino acid digestibility [8]. Our values obtained in  
241 this last study for milk protein, and in the present one for spirulina, are consistent with others  
242 obtained in pigs [17] and humans [4], supporting the reliability of this method to provide a good  
243 proxy of AA digestibility. The second limitation of our study that we did not test for the integrity  
244 of cell walls after sonication. Therefore we cannot exclude absence of significant disruption of  
245 cells. Lastly, the use of intrinsic labeling of dietary protein may overestimate dietary losses due  
246 to tracer recycling in digestive enzymes. However, this phenomenon is limited in the context  
247 of a 6h postprandial test, and furthermore, due to the successive dilutions of <sup>15</sup>N enrichment in  
248 the plasma amino acid pool and then in digestive proteins. Overall, this technique leads to a  
249 minor underestimation of protein digestibility that was assessed as about 1% [18].

250 In conclusion, this study not only provides the first data on spirulina real nitrogen and amino  
251 acid digestibility in rats but also highlights important differences between nitrogen and amino  
252 acid caecal digestibility. We propose that the mean value of caecal individual amino acid  
253 digestibility, which is around 83%, could be used as a reference. This study could not verify  
254 that sonication improved digestibility.

255

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257 author states that there is no conflict of interest.

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260

261 **Author's contribution:** Claire Gaudichon designed the research; Romain Tessier, Juliane  
262 Calvez and Nadezda Khodorova conducted the research; Romain Tessier and Claire  
263 Gaudichon analyzed data; Romain Tessier wrote the article; Juliane Calvez, Nadezda  
264 Khodorova and Claire Gaudichon proofread the article; Romain Tessier and Claire Gaudichon  
265 had primary responsibility for final content. All authors have read and approved the final  
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- 326

327 **Table 1** Meal composition of standard diet and spirulina diet

	Standard diet P14		Spirulina diet	
	g/kg DM	% of energy	g/kg DM	% of energy
Protein	140.0	14.5	193.0	14.9
Soybean oil	40.0	10.4	29.0	8.2
Starch	622.4	63.1	578.0	63.2
Sucrose	100.3	10.9	106.0	12.5
Cellulose	50.0	0.0	48.0	0.0
Minerals <sup>a</sup>	35.0	0.0	34.0	0.0
Vitamins <sup>a</sup>	10.0	1.1	10.0	1.1
Others	2.3	0.0	2.0	0.0

328 Composition in mass (in g/kg of dry matter (DM)) and energy supply (% of energy provided).

329 <sup>a</sup> Minerals and vitamins were formulated from AIN-93M.

330 **Table 2** Amino acid composition of spirulina protein

Spirulina protein			
Indispensable amino acid		Dispensable amino acid	
Histidine	1.41	Alanine	8.22
Isoleucine	5.79	Arginine	7.05
Leucine	8.78	Asx	10.73
Lysine	4.67	Cysteine	1.28
Methionine	1.66	Glx	14.13
Phenylalanine	4.76	Glycine	4.88
Threonine	5.36	Proline	3.95
Valine	6.25	Serine	5.43
		Tryptophan	0.80
		Tyrosine	4.86

331 Amino acid composition is expressed in percentage of all amino acids. Asx: aspartate and  
 332 asparagine. Glx: glutamate and glutamine.

333 **Table 3** Dietary nitrogen recovered in gastrointestinal contents as well as caecal and fecal  
 334 nitrogen digestibility in rats 6 hours after ingestion of <sup>15</sup>N control or sonicated spirulina

	Control Spirulina	Sonicated Spirulina	P <sub>value</sub>
<sup>15</sup> N recovery (% of dietary N ingested)			
Stomach	0.98 ± 0.24	0.15 ± 0.03	<0.01
Small Intestine	0.81 ± 0.10	0.62 ± 0.10	0.19
Ileum	0.31 ± 0.09	0.45 ± 0.12	0.36
Caecum	9.43 ± 0.97	9.70 ± 0.79	0.83
Colon	4.88 ± 0.64	3.20 ± 0.42	<0.05
Nitrogen digestibility (%)			
Caecal digestibility (%)	90.3 ± 0.9	89.9 ± 0.8	0.75
Fecal digestibility (%)	85.4 ± 0.9	86.7 ± 1.0	0.36

335 Digestibility is expressed in percentage of dietary nitrogen ingested. Values are means  $\pm$   
 336 SEMs, n = 13 for CS group and n = 11 for SS. Differences were tested with an unpaired t-test.

337

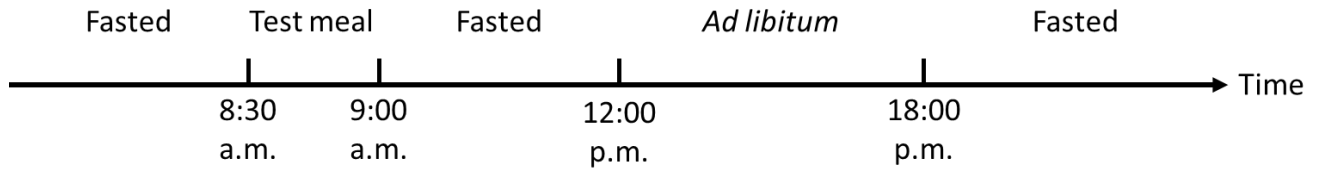
338 **Table 4** Caecal amino acid digestibility of spirulina determined from  $^{15}\text{N}$  enrichment in rats 6 h  
 339 after ingestion of control or sonicated spirulina

	Control Spirulina	Sonicated Spirulina	p value
Indispensable amino acid caecal digestibility (%)			
Leucine	82.3 $\pm$ 1.4	80.7 $\pm$ 1.6	0.48
Isoleucine	82.8 $\pm$ 1.5	82.5 $\pm$ 1.4	0.88
Lysine	85.6 $\pm$ 1.0	86.0 $\pm$ 1.2	0.81
Methionine	89.4 $\pm$ 1.0	87.8 $\pm$ 1.3	0.32
Phenylalanine	82.9 $\pm$ 1.2	83.7 $\pm$ 1.2	0.63
Threonine	85.6 $\pm$ 1.5	82.5 $\pm$ 1.8	0.20
Valine	80.8 $\pm$ 1.4	80.0 $\pm$ 1.8	0.72
Mean	84.2 $\pm$ 0.6	83.3 $\pm$ 0.6	0.29
Dispensable amino acid caecal digestibility (%)			
Alanine	81.9 $\pm$ 1.4	80.1 $\pm$ 1.8	0.43
Glycine	82.0 $\pm$ 1.5	79.3 $\pm$ 1.8	0.25
Glx	83.0 $\pm$ 1.3	82.5 $\pm$ 1.6	0.82
Proline	87.4 $\pm$ 1.0	87.0 $\pm$ 1.0	0.75
Serine	77.9 $\pm$ 1.9	77.3 $\pm$ 1.6	0.82
Tyrosine	83.8 $\pm$ 1.4	83.4 $\pm$ 1.4	0.85
Mean	82.6 $\pm$ 0.7	81.6 $\pm$ 0.7	0.28
Mean (all AA)	82.8 $\pm$ 1.3	81.6 $\pm$ 1.5	0.57

340 Caecal amino acid digestibility is expressed in percentage of amino acid ingested. Glx:  
 341 glutamate and glutamine. Values are means  $\pm$  SEMs, n = 13 for CS group and n = 11 for SS.  
 342 Differences between groups were tested using an unpaired t test.

343 **Figure Caption**

344 **Fig. 1** Schematic representation of feeding pattern during the habituation to the small test  
 345 meal.



346

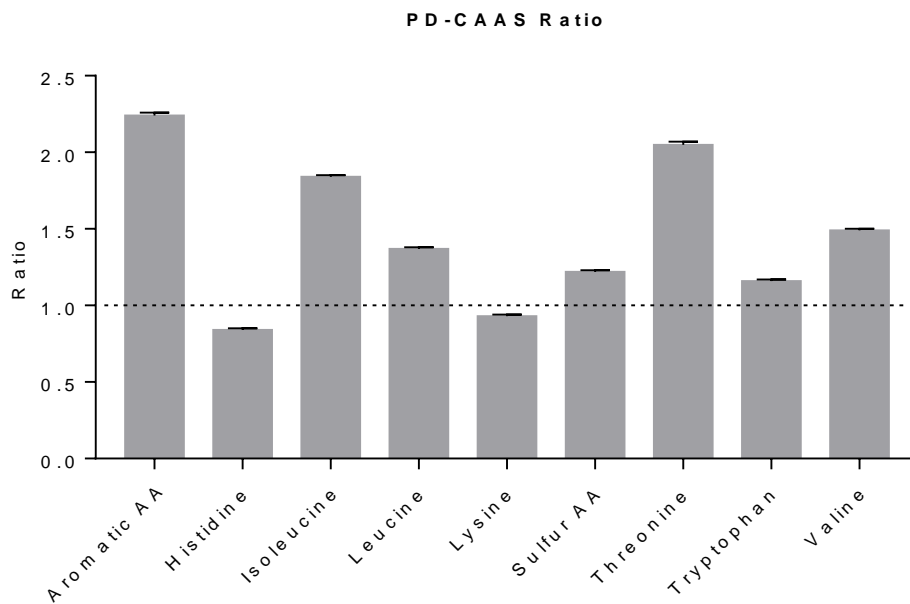
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351 **Fig. 2** Protein Digestibility Corrected Amino Acid Ratio of spirulina. Sulfur AA are methionine  
 352 and cysteine. Aromatic AA are tyrosine and phenylalanine. N conversion factor used: 6.25.  
 353 Values are means  $\pm$  SEMs, n=24



354