

1 Digesta passage time, digestibility and total gut fill in captive Japanese

2 macaques: the effects of food type and food intake level

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18

19 **Abstract**

20           Digestion is an important process in understanding the feeding ecology  
21 of animals. We examined digesta passage time, digestibility, and total gut fill in  
22 Japanese macaques ( $n = 4$ ) under four diet conditions representing the seasonal  
23 and regional variations in the diets of wild populations to determine the effects of  
24 food type and food intake on these digestive features. Food type was  
25 associated with mean retention time (MRT), digestibility, and total gut fill. Dry  
26 matter intake (DMI) of food was positively correlated with total gut fill but not with  
27 MRT or digestibility. Indigestible DMI, on the other hand, affected MRT  
28 negatively. Thus, when Japanese macaques consume high-fiber foods, MRT  
29 becomes shorter and digestibility is lower than eating low-fiber foods.  
30 Moreover, macaques experience increases in total gut fill when they consume  
31 high-fiber diets or a large amount of food. Japanese macaques may excrete  
32 difficult-to-digest food components quickly: they nevertheless buffer an increase  
33 in food intake by an increase in gut fill. Our study offers new insight into the  
34 relationship between feeding ecology and nutritional physiology in primates by  
35 simultaneously examining the effects of food type and intake level on MRT and  
36 digestibility.

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38 Key words: digesta passage time; mean retention time; digestibility; total gut

39 fill; *Macaca fuscata*

40

## 41 **Introduction**

42

43           Animals must absorb nutrients and energy from the foods they ingest  
44 and digest (Robbins 1983). Since the amount of food they need to eat highly  
45 depends on how efficiently they can digest food, digestibility must be taken into  
46 account to understand energy balance. Plant foods are rich in hard-to-digest  
47 structural carbohydrates such as cellulose and hemicellulose. The relatively  
48 large and/or sometimes compartmentalized gastrointestinal tracts and symbiotic  
49 gut microbes of herbivores enable them to retain such fibrous foods for a longer  
50 time period and to carry out adequate bacterial fermentation. In herbivores,  
51 digestion is a time-dependent process (Clauss *et al.* 2007), and the longer the  
52 ingesta is retained in the tract, the better digestibility becomes (Stevens and  
53 Hume 1998). Mean retention time (MRT) is the most reliable single measure to  
54 evaluate the digesta passage time. Digestibility becomes higher with an  
55 increase in MRT values in ungulates (Illius and Gordon 1992; Clauss *et al.* 2007).  
56 A recent review by Clauss *et al.* (2008) also found the same positive relationship  
57 between MRT and apparent digestibility of neutral detergent fiber (NDF; largely  
58 consisting of hemicellulose, cellulose and lignin) among primates, such as

59 lemurs (Edwards and Ullrey 1999a; Campbell *et al.* 2004), howler monkeys, and  
60 colobus monkeys (Edwards and Ullrey 1999b).

61           There are two types of digestive systems in primates; caeco-colic and  
62 forestomach fermentations (Lambert 1998). Most primate species, including  
63 Japanese macaques, are caeco-colic fermenters with a relatively large colon  
64 and/or enlarged caecum for extended microbial fermentation. The colobines,  
65 like nonruminant herbivorous species such as hippos, tree kangaroos and sloths,  
66 on the other hand, are forestomach fermenters having an enlarged, sacculated  
67 forestomach, which enables the animals to carry out extended microbial  
68 fermentation (Chivers 1994; Chivers and Langer 1994; Stevens and Hume 1998).  
69 In general, caeco-colic fermenting animals feed on foods with relatively  
70 digestible components, whereas forestomach fermenting animals rely on foods  
71 with high-fiber contents (Lambert 1998).

72           Wild primates feed on various kinds of foods, reflecting seasonal and  
73 regional differences. Japanese macaques in different regions adopt different  
74 feeding strategies, and diet composition, activity budget, and home range size  
75 are affected by the differences in food availability (Nakagawa 1997; Hanya 2004;  
76 Tsuji and Takatsuki 2004). For example, Japanese macaques in both

77 warm-temperate evergreen forests and cool-temperate deciduous forests  
78 confront difficulties in meeting caloric and nutritional requirements during winter  
79 (Nakagawa *et al.* 1996). In the evergreen forest of Koshima Island, for example,  
80 the major food resource for macaques during winter is mature leaves. These  
81 leaves contain high levels of fiber and low levels of easily digestible  
82 carbohydrates (Iwamoto 1982), but nevertheless food intake is high enough to  
83 compensate for the low diet quality. In the deciduous forest of Kinkazan Island,  
84 on the other hand, macaques feed mainly on winter buds and tree barks, which  
85 also have a relatively low nutritional quality. These food items are so small that  
86 the macaques fail to increase food intake enough to offset the low diet quality.  
87 Based on observations of feeding behavior and nutritional analysis of their foods,  
88 Nakagawa (1989) revealed that energy intake of Japanese macaques during  
89 winter was not enough to cover maintenance energy requirements. In addition  
90 to consumption of fat accumulated during autumn (Wada *et al.* 1975), Japanese  
91 macaques may have physiological adaptations that enable them to meet  
92 nutritional and caloric needs when they confront such bad food conditions. If  
93 macaques are capable of increasing retention time to prolong time for bacterial  
94 fermentation under a food shortage, for example, they might need less food and

95 thus could save time and energy searching for foods. Alternatively, they might  
96 be able to improve digestive activity by increasing total gut fill in response to  
97 changes in food conditions, as in some rodent species (El-Harith *et al.* 1976; Owl  
98 and Batzli 1998; Naya *et al.* 2005).

99           In this study, we simulated variable food environments that wild  
100 populations would face using captive Japanese macaques to determine the  
101 effects of food type and food intake level on mean retention time (MRT),  
102 digestibility and total gut fill as an index of gut intake capacity. We predict that  
103 MRT becomes longer in response to an increase in indigestible material intake  
104 level to have enough time for fiber digestion and prevent a decrease in  
105 digestibility. We also examined the prediction that total gut fill increases when  
106 the macaques consume more indigestible materials as reported in rodents.  
107 This study offers new insight into the relationship between feeding ecology and  
108 nutritional physiology in primates by simultaneously examining the effects of  
109 food type and intake level on MRT and digestibility.

110

## 111 **Materials and methods**

112

## 113 Study Subjects

114

115 We conducted this study on four individually housed adult male  
116 Japanese macaques (mean 13.6 kg body weight; 10.5 years old) at the  
117 Research Resource Station of the Primate Research Institute, Kyoto University  
118 (Table I). All of the animals were born and raised in captivity. Their usual diets  
119 consist of artificial pellets with moderate-level fibers, along with some fruits and  
120 vegetables such as apples and sweet potatoes. We also give them a piece of  
121 wood so that they can nibble the tree bark. The animals did not have any of  
122 these additional food items during each experiment. We carried out the  
123 experiments from January 21, 2008 to March 29, 2008.

124

## 125 Experimental Diets

126

127 To investigate the effect of NDF content of food on MRT and digestibility,  
128 we used high-fiber (NDF 37.5%; Oriental Yeast Co., Ltd.) and low-fiber diets  
129 (NDF 13.6%, PMI Nutrition International) (Table II). During the first experiment,  
130 we gave the animals a small amount (166 g/day in dry matter (DM), or 180 g/day



131 as fed) of high-fiber pellets to study the effects on MRT and digestibility (Table III).  
132 In the next experiment, we fed them a large amount (230 g DM, 250 g as-fed) of  
133 the same high-fiber pellets. In the following two experiments, we gave the  
134 animals low-fiber pellets in the same amounts (Small: 169 g DM, 180 g as-fed;  
135 Large: 235 g DM, 250 g as-fed). Thus, we set four feeding conditions:  
136 High-Small, High-Large, Low-Small and Low-Large. Low-Large represents the  
137 situation where high-quality food is abundant, whereas High-Small reflects the  
138 worst case scenario that macaques in deciduous forests would face during  
139 winter. Daily dry matter intake (DMI) of High-Small was 166 g and the daily  
140 energy intake was 531 kcal (physiological fuel value; provided by the  
141 manufacturer).

142

### 143 Feeding Trials

144

145 Each time before we started feeding the animals a new type of food, we  
146 set a 3-day introduction period and a 5-day adaptation period. The first 3 days  
147 were to gradually change their diets from the original to the experimental ones.  
148 During the next 5 days, the animals consumed only experimental diets so that

149 we could exclude possible effects of the original diets they had previously had,  
150 although gut microbes may take more than 8 days to adapt to the experimental  
151 diet. We fed the animals twice a day, at 10:00h and at 15:00h, and quantified  
152 the amount of food at the individual level. The animals consumed all of the  
153 food given and water was available ad libitum. We checked body weight of the  
154 animals before and after each experiment to maintain their good health (Table  
155 IV).

156

#### 157 Measurement of Digesta Passage

158

159 We used chromium mordanted onto cell-wall constituents (Cr-CWC;  
160 0.08 g/BW kg) prepared from alfalfa (*Medicago sativa*) as the particle Cr marker  
161 and Cobalt-ethylene diaminetetraacetic acid (Co-EDTA; 0.04 g/BW kg) as the  
162 fluid Co marker (Udén *et al.* 1980; Caton *et al.* 1999). We mixed the marker  
163 dose into a piece of pancake and gave it to the animals at 8:00 on Day 1 of each  
164 trial before their morning meals.

165 We set a wire-mesh sheet on four legs under each cage so that we could  
166 easily separate feces from urine. After marker dosing at 8:00h, we monitored

167 the animals every 2 hours for the first 12 hours, then every 4 hours for the next  
168 12 hours (Day 1 - Day 2). We observed the animals every 6 hours for the next  
169 24 hours (Day 2 – Day 3) and every 8 hours for the last 72 hours (Day 3 – Day 6)  
170 (Sakaguchi *et al.* 1991). After collecting samples, we deep-froze them  
171 immediately at -30 °C, and then vacuum-dried them at 60 °C until reaching a  
172 constant weight. Next, we ground dried samples and stored them in plastic  
173 tubes. For determination of chromium (Cr) and cobalt (Co), we ashed each  
174 fecal sample at 550 °C for 6 hours in a muffle furnace. Then we dissolved the  
175 ashed samples in 0.1 N hydrochloric acid solution. We determined Cr and Co  
176 concentration in the treated sample by atomic absorption spectroscopy (Atomic  
177 absorption spectrophotometer AA-660, Shimadzu, Kyoto).

178

#### 179 Measurement of Digestibility

180

181 We determined apparent digestibility of DM (aD DM) and NDF (aD NDF)  
182 in each trial. To carry out nutritional analysis for each feeding trial, we pooled  
183 all the feces of the last 96 hours, from 8:00h on Day 2 to 8:00h on Day 6, for  
184 each animal (Table III). We did not use feces collected prior to this period as an

185 precaution in addition to the 5-day adaptation period since the marker analysis  
 186 suggested that complete marker excretion may take over 100 hours.

187 To estimate aD DM, we also needed to determine food intake during the  
 188 same period. Since we finished the experiment before AM meal on Day 6, we  
 189 considered the total food intake of each trial as the sum of food given from Day 2  
 190 to Day 5. We determined aD NDF in duplicate from 0.5 g samples using the  
 191 methods of Van Soest *et al.* (1991). After removing crude fat from samples by  
 192 the Soxhlet method with a diethyl-ether extract, we boiled them in an NDF  
 193 solution for 1 hour. We dried the NDF residues and then calculated NDF by  
 194 subtracting the ashed residues from them.

195

## 196 Data Analysis

197

198 We calculated the particle Cr and fluid Co MRT of each animal according  
 199 to the formula of Blaxter *et al.* (1956):

$$200 \quad MRT \ (h) = \frac{\sum_{i=1}^N M_i T_i}{\sum_{i=1}^N M_i} ,$$

201

202 where  $M_i$  is the amount of the marker excreted in the  $i$ th defecation and  $n$  is the

203 total number of defecations. We regarded  $T_i$  as the middle of the sampling  
 204 interval so that the calculated MRT would become a better indication of the true  
 205 MRT without frequent sampling (Van Weyenberg *et al.* 2006).

206 We used the following formula for apparent digestibility (Robbins 1983):

207

$$208 \quad aD (\%) = \frac{\text{Amount consumed} - \text{Fecal excretion}}{\text{Amount Consumed}} \times 100$$

209

210 ,based on an average daily food intake and excretion over 4 days.

211 We estimated the amount of Indigestible DMI ( $\text{g/kg}^{0.75}/\text{d}$ ) using the  
 212 following formula:

$$213 \quad \text{Indigestible DMI} = \text{DMI} - (\text{DMI} \cdot A)$$

214 where  $A$  is the fractional digestibility of the diet.

215 We calculated total gut fill of the animals as follows (Holleman and White  
 216 1989):

$$217 \quad V = V_N + \frac{V_N \cdot A}{2(1 - A)}$$

218

219 where  $V_N$  is the indigestible material fill. Of the two formulae provided by

220 Holleman and White (1989), we chose the equation based on the assumption

221 that absorption of ingested food would occur linearly with time. We did not take  
222 the fluid Co marker MRT into account since the marker used in this calculation  
223 must be representative of solid ingesta (Holleman and White 1989).

224 We analyzed MRT values and digestibility using a generalized linear  
225 model (GLM), where food type and intake level were independent variables  
226 (food type: High = 0, Low = 1; food intake level: Large = 0, Small = 1), in R for  
227 Windows version 2.9.2 (2009 The R Foundation for Statistical Computing). We  
228 selected the function with the minimum Akaike Information Criterion (AIC) as the  
229 best-fitted model for each feeding trial. We also analyzed the relationship  
230 between MRT and digestibility as well as that between indigestible DMI and  
231 MRT.

232

## 233 **Results**

234

### 235 **Digesta Passage**

236

237 MRT of both the particle Cr and fluid Co markers became shorter under  
238 high-fiber diets (Fig. 1). The best-fit model selected for the particle Cr included

239 only food type (Cr MRT =  $12.425 * \text{food type} + 35.125$ , AIC = 110.37, df = 15, F =  
240 13.553,  $P = 0.002$ ). Although the best-fit model for the fluid Co included both  
241 food type and food intake level (Co MRT =  $16.038 * \text{food type} + 5.613 * \text{food}$   
242 intake level + 26.456, AIC = 114.77, df = 15, F = 10.131,  $P = 0.002$ ), a model  
243 including only food type also had a small AIC (Co MRT =  $16.038 * \text{food type} +$   
244 29.262, AIC = 115.28, df = 15, F = 16.614,  $P = 0.001$ ), suggesting that food type  
245 had a stronger effect on the MRT of the fluid Co markers. The differences in  
246 MRT for the two diet types were 12 hours for the particle Cr marker (high-fiber:  
247  $35.1 \pm 1.9$  h, low-fiber:  $47.5 \pm 9.3$  h; mean  $\pm$  SD) and 16 hours in the fluid Co  
248 marker (high-fiber:  $29.3 \pm 3.3$  h, low-fiber:  $45.3 \pm 10.6$  h). The amount of food  
249 intake level had no effect on MRT of the both markers.

250 The amount of indigestible material in the four diets was largest in  
251 High-Large, then High-Small, Low-Large, and Low-Small (Fig. 2, Table IV).  
252 MRT of both markers became shorter with increasing indigestible DMI  
253 (Pearson's correlation, Cr:  $r = -0.748$ , df = 14,  $P < 0.001$ , Co:  $r = -0.819$ , df = 14,  
254  $P < 0.001$ , Fig. 3), although this relation was not very clear in the high-fiber diets,  
255 where the animals consumed a relatively large amount of indigestible materials.  
256

## 257 Digestibility

258 Both aD DM and aD NDF measured in the high-fiber diets were lower  
259 than those of low-fiber diets. The best-fit model selected for aD DM included  
260 only food type (aD DM = 26.297 \* food type + 56.897, AIC = 72.50, df = 15, F =  
261 640.73,  $P < 0.0001$ ). Both food type and food intake level showed effects on  
262 aD NDF (aD NDF = 11.469 \* food type – 4.601 \* food intake level + 54.746, AIC  
263 = 100.38, df = 15, F = 13.199,  $P < 0.001$ ), although food type appeared to be  
264 more influential than food intake level (aD NDF = 11.469 \* food type + 54.746,  
265 AIC = 102.34, df = 15, F = 19.234,  $P < 0.001$ ; aD NDF = -4.601 \* food intake level  
266 + 60.48, AIC = 114.56, df = 15, F = 1.4065,  $P = 0.255$ ). High-fiber diets had  
267 more than 25% lower aD DM compared to low-fiber diets ( $56.9 \pm 1.9\%$  and  $83.2$   
268  $\pm 2.3\%$ , mean  $\pm$  SD, respectively), and aD NDF of high-fiber diets was also lower  
269 than that of low-fiber diets ( $53.6 \pm 4.4\%$  and  $62.9 \pm 9.0\%$ , respectively; Table IV).

270 MRT and aD DM were correlated with each other in the trials with  
271 low-fiber diets, but not in those with high-fiber diets (Fig. 4). Since food intake  
272 did not have an influence on MRT values, we pooled all data from the four trials  
273 and then divided them into two groups based on food type. As a result, we  
274 found a significant correlation between the particle Cr MRT and aD DM in the



275 low-fiber diet trials (Pearson's correlation, Cr:  $r = 0.722$ ,  $df = 6$ ,  $P = 0.043$ ),  
276 although the slope of the regression was not steep. There was no such  
277 significant correlation between the fluid Co MRT and aD DM (Co:  $r = 0.695$ ,  $df =$   
278  $6$ ,  $P = 0.056$ ). MRT was not correlated with either aD DM of high-fiber diets  
279 (Pearson's correlation, Cr:  $r = -0.124$ ,  $df = 6$ ,  $P = 0.769$ , Co:  $r = 0.432$ ,  $df = 6$ ,  $P =$   
280  $0.286$ ) or aD NDF of both high-fiber (Cr:  $r = 0.285$ ,  $df = 6$ ,  $P = 0.494$ , Co:  $r =$   
281  $0.385$ ,  $df = 6$ ,  $P = 0.346$ ) and low-fiber (Cr:  $r = -0.036$ ,  $df = 6$ ,  $P = 0.933$ , Co:  $r = -$   
282  $0.084$ ,  $df = 6$ ,  $P = 0.843$ ) diets.

283

## 284 Total Gut Fill

285

286 Total gut fill was associated with both food type and intake level. The  
287 best-fit model for total gut fill included both food type and intake level (Total gut  
288 fill =  $-67.309 * \text{food type} - 28.031 * \text{food intake level} + 142.044$ , AIC = 128.30,  $df$   
289 = 15,  $F = 80.111$ ,  $P < 0.0001$ ). Total gut fill became greater when the animal fed  
290 the diets with either larger amounts or higher fiber levels (Fig. 5).

291

## 292 Discussion

293

294           Two limitations of our study may influence our results. First, we used  
295 NDF rather than acid detergent fiber (ADF) as a parameter of fiber content.  
296 ADF permits more precise comparisons because NDF contains partially  
297 digestible hemicellulose. When two diets given to gorillas contained similar  
298 levels of NDF, the one with high ADF content showed a low digestibility (Remis  
299 and Dierenfeld 2004). However, the high-fiber pellets are also likely to be high  
300 in ADF due to the large difference in NDF levels of the two diets we used (37.5%  
301 in high-fiber; 13.6% in low-fiber, respectively). Second, we had no information  
302 on NDF composition. Hemicellulose is more digestible and lignin is less  
303 digestible, so the higher aD NDF of the low-fiber diet might suggest a high  
304 hemicellulose content, whereas the high-fiber diet contains more lignin. Such  
305 differences in NDF composition may affect aD DM of the two diets. However,  
306 even if that was the case, our result would not be very different because of  
307 distinct differences in both the NDF content and aD DM of the two diets.

308

309 Effects of Food Type

310

311 This study shows that MRT measured in both particle and fluid markers  
312 become much shorter when the Japanese macaques feed in the high-fiber diets.  
313 This tendency has been reported for other primate species including langurs  
314 (Nijboer *et al.* 2007) and chimpanzees (Milton and Demment 1988), and is likely  
315 to be because high-fiber diets contain more indigestible materials, which push the  
316 digesta out to the gut, and thus shorten the MRT.

317 Both the apparent digestibility of dry matter and NDF (aD DM, aD NDF,  
318 respectively) depended on food type. A higher aD DM in the low-fiber diets is  
319 consistent with the previous studies on lemurs (Edwards and Ullrey 1999a),  
320 gorillas (Remis and Dierenfeld 2004), orangutans (Schmidt *et al.* 2009), and  
321 howler and colobus monkeys (Edwards and Ullrey 1999b), and the same  
322 negative correlation exists between aD NDF and fiber contents of diets (Schmidt  
323 *et al.* 2009). Since high-fiber foods contain more indigestible material that  
324 inhibits absorption of nutrients, it is reasonable to find a lower aD DM and aD  
325 NDF.

326 Food type also affects total gut fill of Japanese macaques. Greater  
327 total gut fill in the High-Small diet compared to the Low-Large diet implies a more  
328 significant effect of food type than food intake. The differences in total gut fill

329 among the four diets may indicate the feeding strategy of Japanese macaques in  
330 different food environments. When food contains a lot of fiber, the macaques  
331 can meet their energy requirement either by increasing food intake level or  
332 decreasing MRT down to a point where digestibility might be compromised (Fig.  
333 3). Once their MRT bottoms out due to high indigestible DMI, the macaques  
334 can react by increasing total gut fill in order to prevent further drops in MRT.

335

#### 336 Effect of Food Intake Level

337

338 DMI had little effect on both MRT and digestibility in Japanese macaques.  
339 This result differs from previous studies on herbivores, where MRT became  
340 shorter as DMI increased (Fryxell *et al.* 1994; Clauss *et al.* 2004; Clauss *et al.*  
341 2007), and primates, where a review of studies on 19 species found a significant  
342 negative correlation between MRT and DMI (Clauss *et al.* 2008). This  
343 difference in results may be due to a difference in the range of food intake levels,  
344 which is expressed as relative DMI ( $\text{g/kg}^{0.75}/\text{d}$ ). Relative DMI in this study is  
345 10.2 - 21.0  $\text{g/kg}^{0.75}/\text{d}$  (Table IV), which is smaller than the 11 - 118  $\text{g/kg}^{0.75}/\text{d}$   
346 range of the previous study (Clauss *et al.* 2008). However, the food intake level

347 of wild Japanese macaques usually does not range that wide, and the relatively  
348 small range in food intake level (190 - 299 DM g/d over a 6-month period) among  
349 macaques in the evergreen forest of Koshima (Iwamoto 1982) suggests that the  
350 food intake level in our experiment was reasonable.

351 Indigestible DMI affected the particle Cr marker MRT while food intake  
352 level had little effect on MRT. The more the animals ingested indigestible  
353 materials, the shorter the particle Cr MRT became, although this tendency was  
354 very weak in high-fiber diets since MRT becomes more or less constant once  
355 indigestible DMI reaches a certain level ( $5 \text{ g/kg}^{0.75}/\text{d}$ , approximately) (Fig. 3).  
356 The variation in the particle Cr MRT among the four diets (High-Large,  
357 High-Small, Low-Large, and Low-Small) does not seem to be as great as that  
358 found in indigestible DMI, since there is little difference in MRT between  
359 High-Large and High-Small diets (Fig. 1). This may indicate that the amount  
360 of indigestible material in High-Small is high enough to bring MRT close to the  
361 minimum level, where additional indigestible material in High-Large would no  
362 longer affect MRT.

363 DMI was associated with total gut fill of Japanese macaques.  
364 High-Large diet showed greater total gut intake than High-Small diet, and

365 Low-Large diet showed greater total gut intake than Low-Small diet (Fig. 5).  
366 Japanese macaques may be capable of increasing DMI by increasing total gut  
367 fill when they need to consume a large amount of food. Based on the  
368 combined effects of both food type and intake level on total gut fill, we conclude  
369 that Japanese macaques have a flexible digestive tract that enables them to  
370 deal with different food conditions.

371

## 372 The Digestive Strategy of Japanese Macaques

373

374 The results of this study indicate that aD DM measured in the low-fiber  
375 diets become higher when MRT is longer. However, the correlation was small  
376 since the range of aD DM (79.4 – 85.6%) was much smaller than that of MRT  
377 (Cr: 34.9 – 60.2 h; Co: 31.6 – 59.9 h). Thus, we could not firmly conclude that  
378 aD DM was improved by increased MRT. Compared to low-fiber diets,  
379 high-fiber diets showed a much smaller range of MRT (Cr: 32.6 – 38.6 h; Co:  
380 24.6 – 34.2 h), so we could not examine the influence of MRT on aD DM in the  
381 high-fiber diets.

382 A longer MRT is one way to deal with a high-fiber diet because microbial

383 fermentation of structural carbohydrates requires time. This is true for  
384 leaf-eating primates such as the colobines (Edwards and Ullrey 1999b; Nijboer  
385 *et al.* 2007), but not for Japanese macaque since they have a shorter MRT and  
386 lower aD DM in the high-fiber diets. Our results indicate that the macaques  
387 have a flexible digestive system that allows them to deal with various food  
388 conditions and that there seems to be a gut capacity threshold around 5 g  
389 indigestible DMI/kg<sup>0.75</sup>/d. Below this threshold, the more indigestible materials  
390 the macaques ingest, the shorter MRT becomes. Once they reach the  
391 threshold by consuming high-fiber foods or a relatively large amount of low-fiber  
392 foods, their gut simply expands. These findings suggest that Japanese  
393 macaques ensure MRT never becomes too short; not less than 30 h for particles.  
394 Some rodent species are also known to have a flexible digestive strategy to  
395 meet their energy requirements, increasing the size of the digestive tract in  
396 response to temperature (Naya *et al.* 2005) or diet types (El-Harith *et al.* 1976;  
397 Owl and Batzli 1998; Naya *et al.* 2005), suggesting better digestion due to  
398 increased fermentative activity.

399

400 Implications for the Feeding Ecology of Wild Japanese Macaques

401

402           Our results indicate that Japanese macaques use different food  
403 processing strategies in different food environments. When low-fiber food is  
404 available and indigestible DMI range is low, macaques excrete difficult-to-digest  
405 foods quickly so that they can increase their food intake level. When food  
406 contains a lot of fiber, they can meet their energy requirement either by  
407 increasing food intake level or developing a greater total gut fill.

408           During winter, macaques in evergreen forests can live on mature leaves,  
409 which are low in energy content (Iwamoto 1982). However, since leaves are  
410 large in size, they can stuff themselves with such food in a short feeding time.  
411 In such a food environment, the macaques may meet their energy requirements  
412 by increasing food intake level and/or by developing a greater gut fill. In winter  
413 deciduous forests, on the other hand, few mature leaves are available and  
414 macaques eat mainly winter buds and tree barks, which reduce intake rate and  
415 are difficult to digest (Nakagawa 1989). These animals must therefore survive  
416 even severer food conditions. Macaques in deciduous forests lose their body  
417 weight in winter by consuming fat deposited during autumn (Wada 1975; Wada  
418 *et al.* 1975; Koganezawa 1995). One possible way to deal with such a situation



419 is to increase gut capacity so that they can retain indigestible materials in the gut  
420 long enough to maintain fiber digestibility. Having an almost constant particle  
421 MRT in the high indigestible DMI range may imply such an ability. The fact that  
422 the fecal microflora of wild Japanese macaques in a snowy district was different  
423 from that of captive ones (Benno *et al.* 1987) might also imply that wild Japanese  
424 macaques have another digestive function due to unique intestinal microflora  
425 Thus, our finding might not fully explain feeding adaptations in wild Japanese  
426 macaques and further study of wild macaques is required.

427

428 In conclusion, we found that the digestibility of high-fiber food is lower  
429 than that of low-fiber food regardless of food intake level. Our findings suggest  
430 that Japanese macaques are capable of dealing with various food conditions by  
431 adopting different food processing strategies, under the strong influence of  
432 indigestible DMI level. Macaques excrete difficult-to-digest materials quickly in  
433 the low indigestible DMI range, while they have a constant MRT once  
434 indigestible DMI exceeds a threshold. These results demonstrate the need to  
435 take food type or indigestible DMI into account when comparing MRT and  
436 digestibility within or among primate species.

437

438

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440

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455

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- 571
- 572
- 573

574 **Figure legends**

575

576 Fig. 1 Mean retention time (MRT; h) of two markers by Japanese macaques

577 among four feeding conditions. Box indicates upper and lower quartiles;

578 horizontal line indicates the median; whiskers indicate the range.

579 Fig. 2 Indigestible DMI in four feeding conditions.

580 Fig. 3 The relationship between indigestible DMI and particle Cr MRT.

581 Fig. 4 Relationships between MRT of the markers and apparent digestibility.

582 (a) aD DM (b) aD NDF with the particle Cr marker MRT, (c) aD DM and (d)

583 aD NDF with the fluid Co marker MRT.

584 Fig. 5 Total gut fill in four feeding conditions.

585

586

587 Table I. Details of the animals used in this study

Species	Animal	Born	Age (year)	Origin	Sex	BW (kg)
<i>M. fuscata</i>	1	1995	13	Captive	Male	13.2
<i>M. fuscata</i>	2	1995	13	Captive	Male	16.4
<i>M. fuscata</i>	3	1997	11	Captive	Male	14.7
<i>M. fuscata</i>	4	1999	9	Captive	Male	11.8

588  
589 BW (body weight) was measured on January 20<sup>th</sup>, 2008, a day prior to the start

590 of the first experiment.

591

592 Table II. Major nutritional values of two commercial pellets and energy intake of  
 593 four feeding conditions

	High-Fiber *	Low-Fiber **
Crude protein (%)	18.6	25.9
Crude fat (%)	3.4	4.7
NDF (%)	37.5 <sup>a</sup>	13.6
Crude ash (%)	12.1	5.7
Physiological fuel value (kcal / g)	2.95	3.40
kcal / Large	737.5	850.0
kcal / Small	531.0	612.0

594  
 595 All values are expressed on a fresh basis.

596 NDF (neutral detergent fiber) = hemicellulose, cellulose and lignin

597 Physiological fuel value (kcal / g) = Sum of decimal fractions of proteins, fat and  
 598 carbohydrate x 4, 9, 4 kcal / g, respectively

599 \* Diet for Zoo Animal (ZF), Oriental Yeast Co. Ltd, Tokyo

600 \*\* Certified Primate Diet, PMI Nutrition International, Montana

601 <sup>a</sup>Data from Sakaguchi *et al.* (1999)

602

603 Table III. Mean  $\pm$  SD food intake and feces excretion for each feeding trial

Diet	Food intake		Feces excretion	
	As fed (g)	DM (g)	DM (g)	Frequency
High-Large	1002.8 $\pm$ 2.3	921.5 $\pm$ 2.2	400.2 $\pm$ 14.6	14.0 $\pm$ 0.0
High-Small	723.3 $\pm$ 0.1	664.7 $\pm$ 0.1	284.2 $\pm$ 15.5	11.8 $\pm$ 1.5
Low-Large	1004.7 $\pm$ 0.6	940.0 $\pm$ 0.6	155.4 $\pm$ 20.7	10.3 $\pm$ 1.5
Low-Small	722.9 $\pm$ 0.2	676.3 $\pm$ 0.2	117.7 $\pm$ 16.1	9.0 $\pm$ 1.4

604



605 Food intake is expressed both in fresh (As fed) and dry matter (DM).  
606 Frequency indicates the number of times that we collected fecal samples out of  
607 14 collection times. All values are measured over 96 hours excluding first 24  
608 hours.  
609 High-Large = high-fiber diet in a large amount; High-Small = high-fiber diet in a  
610 small amount; Low-Large = low-fiber diet in large amount; Low-Small = low-fiber  
611 diet in small amount

612 Table IV. Food and indigestible intake, MRT, digestibility and body weight of the animals under four feeding conditions

Diet	Animal	BW		DMI		indigestible DMI		MRT		aD	
		Initial (kg)	Final (kg)	(g/day)	(g/kg <sup>0.75</sup> /day)	(g/day)	(g/kg <sup>0.75</sup> /day)	Cr (h)	Co (h)	DM (%)	NDF (%)
High-Large	1	13.2	13.0	230.1	33.2	96.0	13.9	35.3	31.9	58.3	48.2
	2	16.4	16.4	230.3	28.3	99.0	12.2	34.4	26.3	57.0	55.6
	3	14.7	13.5	230.2	30.7	104.8	14.0	37.1	28.4	54.5	52.7
	4	11.8	10.7	231.2	36.3	100.4	15.8	35.2	24.6	56.6	55.1
High-Small	1	13.0	12.6	166.1	24.3	70.5	10.3	34.2	32.5	57.5	48.9
	2	16.3	15.9	166.1	20.5	69.7	8.6	38.6	34.2	58.0	55.3
	3	14.2	13.7	166.1	22.7	67.4	9.2	32.6	28.8	59.4	53.6
	4	11.6	11.6	166.1	26.4	76.6	12.2	33.6	27.4	53.9	50.1
Low-Large	1	12.8	12.5	235.3	34.8	33.9	5.0	49.9	51.2	85.6	64.9
	2	16.3	16.2	235.2	29.0	38.9	4.8	42.7	41.3	83.4	71.9
	3	14.0	13.7	235.0	32.5	36.5	5.0	48.8	40.5	84.4	68.7
	4	11.2	10.6	234.8	38.4	46.0	7.5	34.9	31.6	80.4	66.6
Low-Small	1	13.0	12.9	169.1	24.7	25.2	3.7	56.0	55.2	85.1	52.3
	2	16.4	16.6	169.1	20.7	26.3	3.2	53.0	51.0	84.5	66.9
	3	13.5	14.1	169.1	24.0	29.3	4.2	60.2	59.9	82.7	65.2
	4	10.7	11.1	169.0	28.6	34.8	5.9	34.9	31.7	79.4	54.9

613

614

615

616

617 Diet: High = high-fiber diet, Low = low-fiber diet, Large = large amount, Small =  
618 small amount; Initial BW = body weight of the animals measured before each  
619 feeding trial; Final BW = body weight measured after each trial; DMI = dry matter  
620 intake; MRT = mean retention time of the two markers (Cr: particle, Co: fluid); aD  
621 DM = apparent DM digestibility; aD NDF = apparent NDF digestibility