Apparent digestibility of captive colobines in relation to stomach types with special reference to fibre digestion

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Abstract

Colobine monkeys are known for the anatomical complexity of their stomachs, making them distinct within the primate order. Amongst foregut fermenters, they appear peculiar because of the occurrence of two different stomach types, having either three ('tripartite') or four ('quadripartite', adding the praesaccus) chambers. The functional differences between tri and quadripartite stomachs largely remain to be explained. In this study, we aim to compare the apparent digestibility (aD) in tripartite and quadripartite colobines. Hence, we measured the aD in two colobine species, *Nasalis larvatus* (quadripartite) and *Trachypithecus cristatus* (tripartite), in two zoos. We also included existing colobine literature data on the aD and analysed whether the aD of fibre components is different between the stomach types to test the hypothesis of whether quadripartite colobines show higher aD of fibre components than tripartite colobines did. Our captive *N. larvatus* specimen had a more distinctively varying nutrient intake across seasons with a larger seasonal variation in aD than that of a pair of *T. cristatus*, which mostly consumed commercial foods with a lower proportion of browse and less seasonal variation. We observed higher aD of dry matter (DM), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the *N. larvatus* specimen, suggesting a higher gut capacity of *N. larvatus* provided by the additional praesaccus forestomach chamber. Based on the analysis of literature data for aD, we also found that quadripartite species achieved higher fibre digestibility at similar dietary fibre levels compared with tripartite species, supporting the hypothesis that the additional gut capacity offered by the praesaccus facilitates a longer retention and hence more thorough microbial fermentation of plant fibre.

Introduction

Primates display a diverse array of digestive adaptations while covering various trophic niches, from folivory to frugivory, gummivory, insectivory, omnivory and nearly exclusive carnivory in some human populations [1, 2]. In particular, Old World monkeys of the subfamily Colobinae are known for the anatomical complexity of their stomachs, making them distinct within the primate order as the only 'foregut fermenters'. Their stomachs are complex and multi-chambered, harbouring a symbiotic microbiome that digest plant fibre and detoxify defensive plant chemicals, possibly allowing them to exploit a diet of leaves in greater quantities than other simple-stomached primates [3, 4]. Because of such anatomical complexity with their folivory, colobines have historically often been considered difficult to maintain healthy under zoo feeding regimens, especially when compared with frugivorous and/or omnivorous primates with simple stomachs [5, 6].

In previous studies, two different types of colobine forestomach have been distinguished. The so-called 'tripartite' type comprises a saccus, tubiform and glandular stomach part; these can be found in all colobines. The 'quadripartite' type has an additional blind sac, or pouch, named 'praesaccus', which is thought to represent an additional chamber. It is found in the genera *Procolobus*, *Piliocolobus*, *Rhinopithecus*, *Pygathrix* and *Nasalis* [7-9]. Notably, genera with a quadripartite stomach are notoriously difficult to maintain and breed in captivity, especially in temperate regions [10-13], compared with species with a tripartite stomach. Therefore, to reduce gastrointestinal disorders and enhance health and survival in captive colobines, identifying their appropriate diet in relation to their digestive physiology is one of the goals for *ex situ* animal management.

The functional differences between tri and quadripartite stomachs, however,

largely remain unexplained. Matsuda, Chapman and Clauss [14] compiled literature data on the natural diet of colobine species to clarify the role of the praesaccus, suggesting that a larger gut capacity provided by an additional praesaccus is an important characteristic by which colobines survive on diets with a particularly high proportion of leaves. Thus, the higher intake capacity for species with quadripartite stomach would be assumed to be detrimental in the case of more digestible (commercial) diets in captivity than those in the wild, thereby leading to malfermentation [5, 6]. Conversely, species with tripartite stomachs might be less susceptible to extreme bouts of malfermentation when fed highly digestible diets because of their relatively reduced intake capacity. Evidently, these speculations remain to be tested.

One approach is to compare the apparent digestibility (aD), i.e. the ratio of the difference of the ingested and faecal nutrients to the ingested nutrients, in tripartite and quadripartite colobines to obtain information relevant for evaluating the digestive ability of the fibre. The aD has previously been quantified in some colobines in comparison to simple-stomached primates, indicating that colobines show higher aD of fibre components, e.g. neutral detergent fibre (NDF) and acid detergent fibre (ADF), than simple-stomached primates, such as *Macaca fuscata* [15], *Alouatta* spp. [16] and *Nomascus siki* [17], did. However, to our knowledge, a study focusing on comparing aD between tripartite and quadripartite colobines has not yet been undertaken.

As a first preliminary approach to this question, we examined the aD of two captive colobine species, *Nasalis larvatus* (quadripartite) and *Trachypithecus cristatus* (tripartite), in two temperate region zoos. We focused on the seasonal difference of their aD to evaluate their digestive capacity throughout the year. Additionally, to test the hypothesis of whether quadripartite colobines show higher aD of fibre components than

tripartite colobines did, we included existing colobine literature data on the aD and analysed whether the aD of fibre components is different between the stomach types.

Materials and methods

Ethics statement

We conducted the feeding experiments of proboscis monkey (*N. larvatus*) and silvered langur (*T. cristatus*) in the Yokohama Zoological Gardens, Zoorasia (approval ID: #256) and Japan Monkey Centre in Japan (approval ID: #2018-016), respectively. Invasive approaches such as capturing, retention and anaesthesia were not performed in this study. The materials are non-invasively collected from animals. This study was approved by the Welfare of Gifu University (approval ID: #17092). All animal experiment procedures were conducted following the Guidelines for Proper Conduct of Animal Experiment (Science Council of Japan, 2006; http://www.scj.go.jp/ja/info/kohyo/pdf/kohyo-20-k16-2e.pdf) and the Guidelines of Animal Research and Welfare of Gifu University (2008; https://www.gifu-u.ac.jp/20150821-12a-experi.pdf).

Digestive trials

In the Yokohama Zoological Gardens, the experiments were performed with one male *N. larvatus* (14 years old) individually housed. Three digestive trials were conducted in different seasons: autumn (3rd–16th September 2017), winter (2nd–15th January 2018) and summer (8th–15th 2018 June 2018). Each trial was composed of two different continuous periods: the acclimatisation (seven days) and sampling (seven days) periods. We were compelled to shorten the acclimatisation (8–12 June 2018) and sampling (13–

15 June 2018) periods due to heavy rain that soaked animal faeces and leftover leaves in the cage. The animal was fed a mixed diet of leaves, vegetables and commercial pellets (Table S1) two times daily. Water was freely available at all times.

In the Japan Monkey Centre, the trials were performed with two adult male *T. cristatus* (16 and 17 years old) housed together. Two experiments were conducted in different seasons: summer (30 July to 12 August 2018) and winter (14–27 February 2019) with applying to the same design as in *N. larvatus*, i.e. two continuous periods. The animals were fed a mixed diet of leaves, vegetables and commercial pellets (Table S2) three times daily. The amounts of each feed were the same between trials across the seasons. Tree leaves were fed with branch and twigs and fed once a day at noon, and the other feeds were fed in the morning and evening.

We measured the body weight of all animals before and after the sampling periods in each experiment (*N. larvatus*: Digital Platform Scale, DP-8100, Yamato, Japan; *T. cristatus*: SD75LJP, OHAUS Corporation, USA).

Sampling procedures

Feed intake was quantitatively recorded over seven sampling days. Each food item was weighed before it was offered to the animals and left in their enclosures until the next feeding session. Table S1–2 shows the mean (±standard error) daily amount of offered food per animal. All leftover food was removed, and the enclosure was cleaned before fresh food items were offered. All food items and leftovers were weighed with accuracy of 2 g (browse, UDS-500N, Yamato, Japan) or 1 g (others, UH-3201, A&D Company, Japan). Leftover weights were adjusted by deriving a desiccation factor from the measured moisture lost from similar sets of food placed in a desiccation pan in an area adjacent to the primate enclosures. For two *T. cristatus* at Japan Monkey Centre housed

together, individual feed intake and faeces output were calculated as the average of total measures divided by two.

We collected equal amounts of each feedstuff at every feeding time during the sampling period and stored them in a refrigerator (4 °C) or freezer (−20 °C) for nutritional analysis. We also collected all faeces shortly before every feeding time and immediately preserved them in the freezer. We mixed each feed sample and then collected 100 g of each leaf and all amounts of other foods as representative samples. We mixed all faeces with 1 ml/100 g of 10% formalin solution and preserved 500 g faeces as a representative sample [18].

After measuring the fresh weight of representative samples, in the case of *N. larvatus*, we lyophilised leaf and faeces samples using a freeze-dryer (DC400/800, Yamato, Japan), except for other feedstuff samples using an air-forced dry oven (DKM812, Yamato, Japan) for 48 h at 60°C. Note that we mashed soybean and peanuts in a mortar due to high-fat content and then washed and soak with ether to extract fat. After fat extraction, the soybean and peanuts were dried using an air-forced dry oven (DKM600, Yamato, Japan) for 3 h at 60°C. In the case of *T. cristatus*, we dried all feedstuff and faecal samples using an air-forced dry oven (DKM812, Yamato, Japan) for 48 h at 60°C.

We ground leaf and faecal samples using a Wiley mill through a 2-mm screen and ground other feed samples using a coffee mill to avoid heat denaturation of sugars in the feeds.

Estimation of apparent digestibility (aD)

We used the average daily feed intake and faecal output during the sampling term to estimate the aD in each trial. We analysed the dry matter (DM), crude ash (CA), crude

protein (CP) and acid detergent fibre (ADFom) in accordance with AOAC 930.15, 942.05, 990.03 and 973.18, respectively [19]. We then analysed neutral detergent fibre (aNDFom) according to the method of van Soest, Robertson and Lewis [20]. Detergent fibre data are presented without residual ash. We calculated aD of each nutrient (N) according to the following equation: aD_N (%) = ($N_{feed\ intake}$–N_{feces}) / $N_{food\ intake}$ × 100.

Literature data and analysis

With our new experimental data, as shown in Table 1, we used published aD data of seven colobine species, including four tripartite species (13 datasets) [15, 16, 21-24] and two quadripartite species (five datasets) [16, 17, 25] to compare digestive capacity between the stomach groups. These published data included total feed and fibre intake and aD of DM, NDF and ADF. We examined the relationship between the proportion of NDF intake in the diet and aD in DM, NDF and ADF and slope and intercept were estimated using linear regression analysis. The analysis of covariance served to compare the slopes and intercepts of regression lines between the stomach types. Note that we could not conduct the same statistical analysis for the aD of CP because of the lack of published data. Additionally, because Nijboer et al. [26] analysed only crude fibre as fibre contents and Coudrat and Cabana [17] did not specify DM contents in diets and faeces with the total intake (see Table 1), we could not include these data in our analysis. All statistical analyses were performed in Spyder (Python 3.7).

Results

Digestive trials

The body weight of *Nasalis larvatus* in autumn, winter and summer was 17.3, 18.9 and 17.8 kg, respectively, shortly before beginning of the sampling periods. The

composition and amount of the diet were different amongst trials (Table S1). The ratio of leaves/other feed intake were 7.4:1, 3.3:1 and 3.5:1, in autumn, winter and summer. Browse species generally contained more fibre than other feeds (Table. S3–5), possibly leading to different proportion of ADFom in the diet in autumn, i.e. 33.3%, 29.4% and 30.1% of DM in autumn, winter and summer, respectively. There was notable seasonal variation in the nutritional composition of the browse species (Table S3–5). The DM and ash contents of laurel (*Machilus thunbergii*) in winter were 5%–10% higher than those in summer and winter. The aNDFom and ADFom of each browse species in winter were 5%–10% lower than those in summer and autumn. On the other hand, nutrient contents of fruits, vegetables, beans, starchy foods and commercial products in *N. larvatus* did not differ between the seasons (Table S3–5). The aD of DM was 69.9%, 79.6% and 73.7% in autumn, winter and summer, respectively (Table 2). Likewise, the aD of CP, aNDFom and ADFom varied amongst the three seasons (Table 2).

The body weight of the two *T. cristatus* was 7.6 and 7.6 kg in summer and 6.8 and 7.7 kg in winter shortly before beginning of the sampling period. There was strong seasonality in the nutritional composition of their browse (Table S6–7). The DM and ash contents in each browse species in *T. cristatus* were higher, and CP, aNDFom and ADFom were lower in winter than those in summer. The other diet items, i.e. fruits, vegetables, starchy foods and commercial product, did not differ between the two seasons (Table S6–7). The aD of DM, ash and ADFom did not differ between the two seasons; only the aD of CP and aNDFom changed between the two seasons (Table 2).

Literature data

Apparent digestibility

There was a significant negative relationship between the aD of DM and the NDF

content of the ingested diet of six colobine species ($r = -0.770$, $p < 0.001$). The difference in the regression slopes and intercepts for aD of DM vs. NDF content of the ingested diet between the stomach types was not significant (Fig. 1; slope, $t = 1.079$; $p = 0.293$; intercept, $t = 1.092$, $p = 0.287$). There was also a significant negative relationship between the aD of NDF or the aD of ADF and the NDF content of the ingested diet of six colobine species (NDF, $r = -0.459$, $p = 0.021$; ADF, $r = -0.439$, $p = 0.028$). The differences in the regression intercepts for both aD of NDF and aD of ADF vs. NDF content was significant (NDF, $t = -2.559$, $p = 0.018$; ADF, $t = -2.487$, $p = 0.021$) between the stomach types (Fig. 1), while the slopes were not different (NDF, $t = 0.267$, $p = 0.792$; ADF, $t = 0.152$, $p = 0.881$).

Discussion

As expected, we confirmed the variation in the aD because of the nutrient differences of animal diets across the seasons. Each browse species nutrient components seasonally changed, as with deciduous tree species in North American zoos [27], whereas those of commercial food items were stable (Table S3–7). Consequently, the aD of *N. larvatus* had a larger seasonal variation, with more distinctively varying nutrient intake across seasons, than that of *T. cristatus*, which mostly consumed commercial foods with a lower proportion of browse.

The most important factor influencing the seasonal variation in aD in *N. larvatus* is likely the fibre intake, i.e. the aNDFom and ADFom contents of the diet. The aD of DM, aNDFom and ADFom in *N. larvatus* clearly changed across seasons: aD DM by 9.1%, aD aNDFom by 11.2 % and aD ADFom by 5.1%. On the other hand, aD was relatively stable between the two different seasons in *T. cristatus*, although the total DM intake in winter was slightly higher than that in summer. As in the present study,

Edwards and Ullrey [16] noted that the aD of DM in three colobine species (*Colobus guereza*, *Pygathrix nemaeus* and *Trachypithecus francoisi*) decreased with an increased fibre level in the diet, supporting that aD is affected by a variation in food composition and seasonally varying nutrient contents in feeds. Thus, evaluating the nutrient diet and aD of captive colobines living in temperate regions throughout the year may contribute to their health management and predict intake requirements across different diets and seasons.

Comparison of the nutrient composition (especially fibre) of faeces in free-ranging and captive individuals has been proposed to obtain information relevant for the improvement of diets of colobines [10]. *Nasalis larvatus* in our study had faecal NDF contents (35%–41% in DM) that were higher than those reported in other captive conspecifics, i.e. 17% [25] (mean of two different values), but lower than those of free-ranging ones, i.e. 53%–70% [10]. Although faecal NDF contents of free-ranging *T. cristatus* are not available, those in our study (30%–31%) were comparable to other closely related species in captivity, i.e. 37% (mean of six different values in *Trachypithecus auratus*) [22] and 31% (mean of three different values in *T. francoisi*) [21], although still far lower than those reported for free-ranging *N. larvatus*. Altering the diets of captive colobines to include more fibre, comparable to those of free-ranging ones, may be recommendable.

In the present study, the NDF level of the total DM intake was much higher in the quadripartite species *N. larvatus* (35.6%–37.9%) than in the tripartite species *T. cristatus* (12.9%–15.0%), because of a much higher proportion of browse fed to the former. This zoo practice may stem from the impression that quadripartite species are generally more difficult to maintain in captivity; therefore, more effort is undertaken to provide them with feed items considered natural for them, mainly browse.

Correspondingly, we observed a higher DM intake (% BW, shown in Table S3–5) in *N. larvatus* compared with those in *T. cristatus* that could be interpreted as compensation for the higher fibre levels. However, regardless of the higher fibre and intake levels, we observed higher aD in DM, aNDFom and ADFom (Table 2) in the *N. larvatus* specimen. This observation is most parsimoniously explained by a higher gut capacity in the proboscis monkey, provided by the additional praesaccus forestomach chamber. Typically, a higher relative food intake leads to shorter digesta retention times and can also compromise digestibility [28, 29, cf. also Fig. 2]. However, a higher gut capacity can mitigate this effect, and this may be the main adaptive value of the praesaccus in quadripartite species [14].

The results of our analysis of literature data for aD support the idea of such functional differences between the stomach types. There was no difference in how dietary fibre content influenced the overall DM digestibility, which is, in most of the diets used in these studies, most likely dominated by the digestion of non-fibrous carbohydrates and protein. However, stomach type had a significant effect on how dietary fibre levels affected the digestibility of fibre itself. Here quadripartite species achieved higher fibre digestibility at similar dietary fibre levels than tripartite species did, suggesting that the additional gut capacity offered by the praesaccus facilitates longer retention and hence more thorough microbial fermentation of plant fibre. Furthermore, one notable tendency was that the quadripartite species, especially *N. larvatus*, achieved a higher digestibility, driving the difference between the two stomach types. In this species, regurgitation and remastication have been observed in the wild [30], and a smaller faecal particle size of *N. larvatus* compared with other colobines has been reported [31]. Because particle size reduction via chewing is one of several key factors affecting digestibility, we cannot determine whether the difference in

digestibility observed here is related to stomach type or chewing efficiency. Ideally, in future studies, similar diets should be employed as this will allow meaningful comparison of faecal particle size; chewing behaviour should also be observed.

Unfortunately, the current available data on digesta retention times in colobine species does not allow testing for a general difference between tripartite and quadripartite species (Fig. 2). Between species, retention times need to be compared in relation to the food intake level [28, 32]. However, the ranges of intake level in published studies hardly overlap between the stomach groups, making a reasonable comparison impossible. To test whether quadripartite species achieve longer digesta retention because of a higher gut fill, comparative studies with different species on a similar (possibly browse-dominated) diet would be required where intake, digestibility and digesta retention are assessed in the same experiment, additionally facilitating the calculation of gut fill [33, 34]. Ideally, such a study would also address the problem of the very limited sample size of the present experiments. Until such a study is performed, our results must be preliminary considered, delivering plausible hypotheses.

It should be noted that we cannot exclude the possibility of the effects of specific fibre-digesting bacteria in the presaccus in quadripartite species. So, far, only a few analyses of the forestomach microbiome are available for colobines. Although the recent developments in sequencing technology describe the foregut microbiome in some colobines, e.g. *N. larvatus* [35] and *Rhinopithecus roxellana* [36], the function of these bacteria species has not been evaluated. However, there is currently also no reason to assume that the praesaccus should harbour a fundamentally different microbiome from the saccus. Detailed studies about differences in the microbiome at different forestomach locations, as available in ruminants [e.g. Zeitz et al. 37], do not exist for colobine monkeys to date. In contrast to the detailed knowledge about the differential

function of individual forestomach sections in ruminants and camelids [e.g. Clauss et al. 38], there is, to date, no indication of the differential function of the forestomach compartments of non-ruminant foregut fermenters [39] beyond the provision of sheer fermentation chamber capacity, and this may well also apply to colobines.

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Author contributions

SH, IM, MC and MY conceptualised the idea and drafted the manuscript; SH performed the feeding trials; SH, IM and MY performed and interpreted the statistical analysis; SS, TF and TH arranged the sampling in the zoos and MY organised the projects. All authors contributed to the final version of the manuscript.

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Supporting information

Table S1. Feed composition, and offered and leftover amounts (g as fed/day) in each digestion trial of an adult male of *Nasalis larvatus*

Table S2. Feed composition, and offered and leftover amounts (g as fed/day/two animals) in each digestion trial of two adults of *Trachypithecus cristatus*

Table S3. Nutrient contents in the feeds and DM intake of an adult male of *Nasalis larvatus* in September 2017 (autumn)

Table S4. Nutrient contents in the diet and DM intake of an adult male of *Nasalis larvatus* in January 2018 (winter)

Table S5. Nutrient contents in the diet and DM intake of an adult male of *Nasalis larvatus* in June 2018 (summer)

Table S6. Nutrient contents in the diet and DM intake of two adults *Trachypithecus cristatus* in August 2018 (summer)

Table S7. Nutrient contents in the diet and DM intake of two adults of *Trachypithecus cristatus* in February 2019 (winter)

Table 1. Summary of colobine apparent digestibility in the previous studies

Colobus monkey		Feed stuff				Apparent digestibility			Reference
Stomach compartments	species	Browse	Fruits Vegetable	Artificial Food	Others*	DM	NDF	ADF	
3	*Trachypithecus francoisi*			✓ (15ADF)		80.3	77.0	80.1	Edwards and Ullrey [16]
				✓ (30ADF)		78.7	74.3	56.2	
	Colobus guereza	✓	✓	✓		65	56	56	Nijboer et al. [22]
		✓	✓	✓		68	63	56	
			✓	✓		83 (82-84)	68	68	Oftedal, Jakubasz and Whetter [23]
			✓	✓	✓	87.1 ± 0.1	81.3 ± 1.0	69.3 ± 0.5	Watkins, Ullrey and Whetter [24]
				✓ (15ADF)		82.4±3.0	79.3 ± 5.3	82.3 ± 4.8	Edwards and Ullrey [16]
				✓ (30ADF)		76.7 ± 3.5	75.7 ± 7.7	76.9 ± 12.8	
		✓	✓	✓		58.9	46.6	38.5	Nijboer et al. [21]
		✓	✓	✓		64.7	62.2	56.1	
		✓	✓	✓		74.0	68.0	65.3	
	Trachypithecus auratus	✓	✓	✓	✓	68	-	-	Nijboer et al. [26]
		✓		✓	✓	59	-	-	
	Trachypithecus cristatus			✓		75.7 ± 5.5	68.9 ± 5.8	61.6 ± 7.5	Sakaguchi et al. [15]
	Trachypithecus obscurus	✓	✓	✓	✓	91	70	53	Nijboer et al. [22]
		✓	✓	✓	✓	84	77	54	
		✓	✓	✓	✓	78	74	65	
		✓	✓	✓	✓	78	64	60	
4	*Nasalis larvatus*	✓	✓	✓	✓	88.3	86.4	85.0	Dierenfeld, Koontz and Goldstein [25]
		✓	✓	✓	✓	88.8	86.2	86.0	
	Pygathrix nemaeus			✓ (15ADF)		76.2	66.5	66.6	Edwards and Ullrey [16]
				✓ (30ADF)		73.9	69.8	67.6	
		✓	✓			-	89.2	83.9	Coudrat and Cabana [17]

*Others contain non-fibrous carbohydrate (rice, bread, sweet potato, cereal and Saint John's bread), animal protein (boiled egg, meat and mealworm) and vegetable

protein (tofu)

Table 2. DM intake, faecal output and apparent digestibility (aD) in *Nasalis larvatus* and *Trachypithecus cristatus*

	Nasalis larvatus			*Trachypithecus cristatus*	
	2017 Sep (Autumn)	2018 Jan (Winter)	2018 Jun (Summer)	2018 Aug (Summer)	2019 Feb (Winter)
Body mass (kg)	17.3	18.9	17.8	7.6 / 7.6	6.8 / 7.7
DM intake (g DM/day/animal)	702.0	654.9	664.3	146.0	133.3
Fecal output (g DM/day/animal)	172.3	109.1	102.8	17.6	16.4
Ingested diet					
OM (%DM)	91.3	90.6	92.6	93.8	93.4
CP (%DM)	11.7	11.7	11.9	7.3	7.9
αNDFom %DM)	37.9	35.3	37.4	12.9	15.0
ADFom (%DM)	33.3	29.1	30.1	10.3	12.7
Feces					
OM (%DM)	89.3	88.5	87.4	88.4	88.3
CP (%DM)	17.3	14.9	19.0	26.3	25.0
αNDFom %DM)	39.4	41.4	34.5	31.1	30.0
ADFom (%DM)	34.7	40.2	39.8	23.3	28.2
aD DM (%)	75.5	83.0	84.5	87.9	87.7
aD OM (%)	76.0	83.7	85.4	88.6	88.3
aD CP (%)	63.8	78.8	75.3	56.4	61.1
aD αNDFom (%)	74.5	80.4	85.7	70.8	75.4
aD ADFom (%)	74.4	77.0	79.5	72.7	72.6

DM, dry matter; OM, organic matter; CP, crude protein; aNDFom, neutral detergent fibre; ADFom, acid detergent fibre

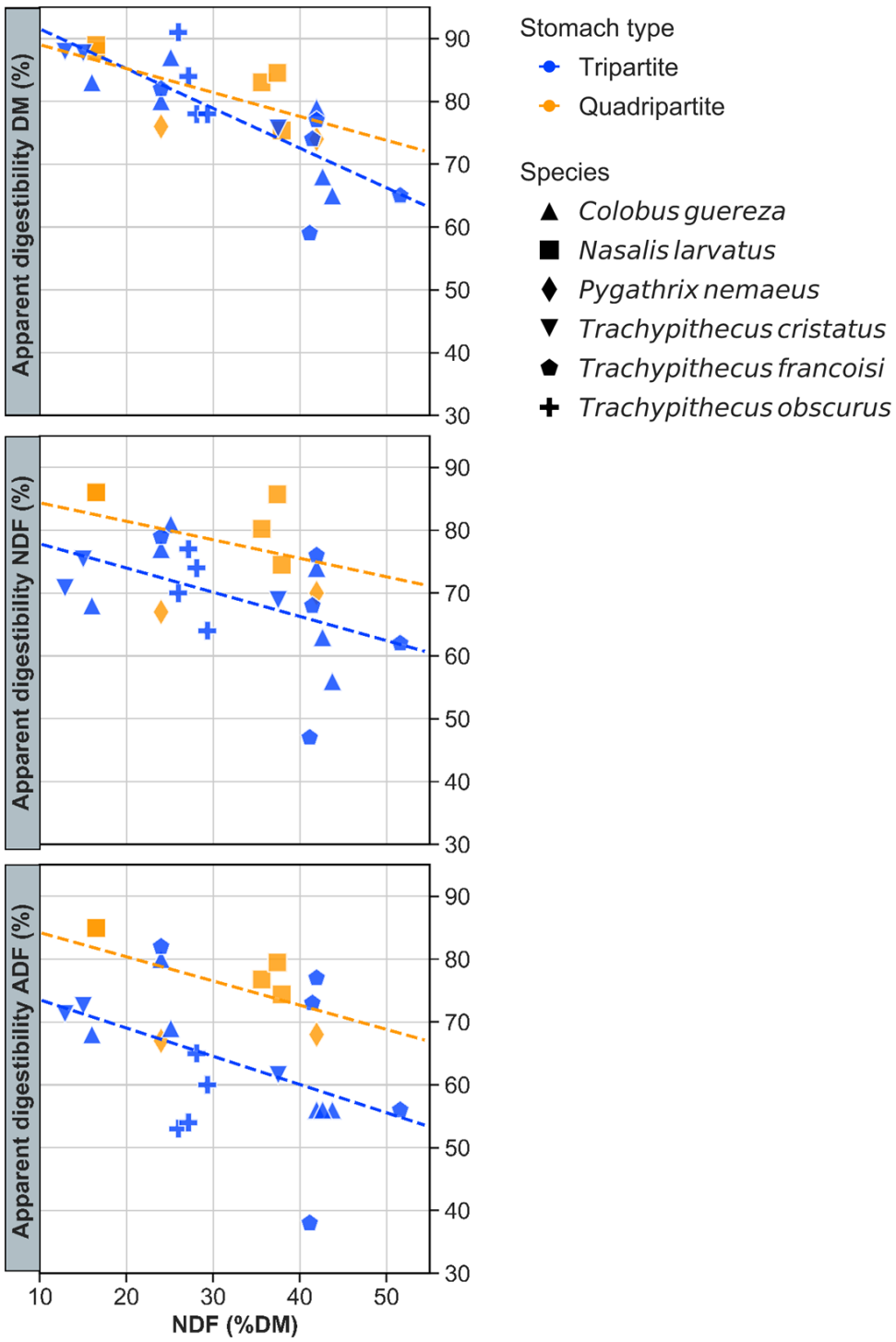

Fig 1. Relationship between apparent digestibility of dry matter (DM, %), neutral detergent fibre (NDF, %) or acid detergent fibre (ADF, %) and neutral detergent fibre intake (%) in six colobine species: *Colobus guereza* [16], *Nasalis larvatus* [25],

Pygathrix nemaeus [16], *Trachypithecus cristatus* [15], *Trachypithecus francoisi* [16, 21] and *Trachypithecus obscurus* [22]. Note that species and stomach type, i.e. tri or quadripartite, are indicated in different shape and colour symbols, respectively.

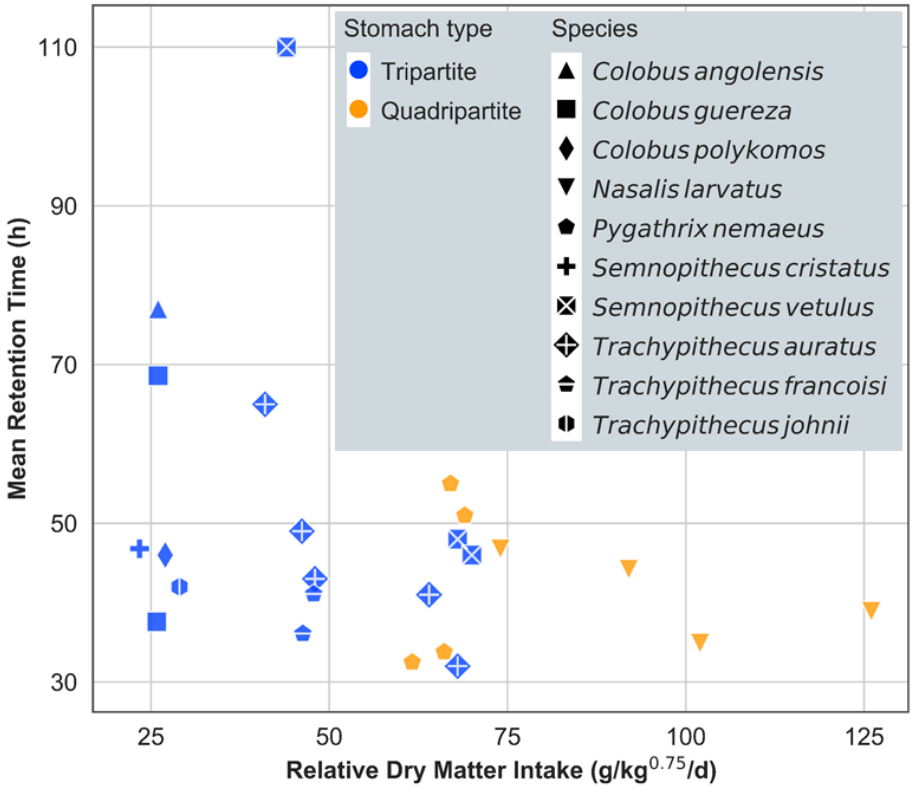

Fig. 2. Relationship between mean retention time (h) and relative dry matter intake ($g/kg^{0.75}/d$) in 10 colobine species: *Colobus angolensis* [40], *Colobus guereza* [16], *Nasalis larvatus* [41], *Pygathrix nemaeus* [16], *Semnopithecus cristatus* [15], *Semnopithecus vetulus* [42], *Trachypithecus auratus* [26], *Trachypithecus francoisi* [16] and *Trachypithecus johnii* [40]. Note that species and stomach type, i.e. tri or quadripartite, are indicated in different shape and colour symbols, respectively.

Table S1 Feed composition, and offered and leftover amounts (g as fed/day) in each digestion trial of an adult male of *Nasalis larvatus*

		2017 Sep (Autumn)		2018 Jan (Winter)		2018 Jun (Summer)	
		Offered*1	Leftover*1	Offered*1	Leftover*1	Offered*1	Leftover*1
Browse*2	Yoshino cherry (*Prunus yedoensis Matumura*)	1551±120	996±128	-	-	1537±420	1114±287
	Bamboo-leaf oak (*Quercus myrsinifolia*)	1708±221	1441±190	2111±279	1930±276	-	-
	Chinquapin (*Castanopsis sieboldii*)	1611±146	1405±124	969±110	358±264	-	-
	Laurel (*Machilus thunbergii*)	1077±126	846±99	2583±426	2113±373	3317±931	2682±619
	Glossy privet (*Ligustrum lucidum*)	1339±130	1177±108	1040±73	894±77	1799±626	1637±595
	Willow (*Salix spp.*)	761±139	452±96	-	-	-	-
	Hibiscus (*Hibiscus spp.*)	-	-	187±22	58±8	-	-
	Japanese spindletree (*Euonymus japonicus*)	-	-	768±35	571± 39	758±88	382±131
Fruit	Apple	129±6	0	163±1	3±2	167±5	0
Vegetable	Carrot	123±2	1±1	125±1	93±2	122±13	14±7
	Green bean	124±2	2±1	173±3	101±5	158±2	9±2
	Broccoli	108±2	0	148±3	3±2	122±12	0
	Asparagus	104±1	0	101±1	4±1	77±8	2±2
	Cucumber	199±4	5±5	121±6	3±3	97±5	0
Beans	Soy bean	31±1	0	41±1	0	37±2	0
	Peanuts	25±1	6±1	73±2	26±1	61±4	29±0
Commercial product	Primate L/S biscuit banana (Mazuri)	16±1	2±0	141±2	2±1	58±0	0

*1 Weight unit is X ± SE g, as fed/day/animal.

*2 We measured the weight of the whole branch, including leaves and twigs.

Table S2 Feed composition, and offered and leftover amounts (g as fed/day/two animals) in each digestion trial of two adults of *Trachypithecus cristatus*

		2018 Aug (Summer)		2019 Feb (Winter)	
		Offered*1	Leftover*1	Offered*1	Leftover*1
Browse*2	Bamboo-leaf oak (*Quercus myrsinifolia*)	300±0	269±5	300±1	280±4
Fruit	Apple	600±1	81±16	600±0	1±1
	Banana peel	80±0	3±1	80±0	0
Vegetable	Carrot	60±1	1±1	60±0	0
	Cabbage	900±0	69±17	901±0	9±2
Starchy food	Sweet potato	320±0	0	321±0	0
Commercial product	Leaf-eater primate diet - Mini-biscuit (Mazuri)	20±0	0	20±0	0

*1 Weight unit is X ± SE g FM/day.

*2 We measured the weight of the whole branch, including leaves and twigs.

Table S3. Nutrient contents in the feeds and DM intake of an adult male of *Nasalis larvatus* in September 2017 (autumn)

	DM (%)	Ash (% DM)	CP (% DM)	aNDFom (% DM)	ADFom (%DM)	DMI (g DM/day)	DMI*1 (%)	DMI*2 (%)	DMI*3 (g DM/kg$^{0.75}$/day)
Yoshino cherry (*Prunus yedoensis Matumura)*	34.15	8.06	9.24	29.47	28.28	189.6	-	27.0	22.4
Bamboo-leaf oak (*Quercus myrsinifolia*)	41.95	8.38	10.90	49.96	36.26	111.8	-	15.9	13.2
Chinquapin (*Castanopsis sieboldii*)	39.39	6.65	9.96	49.47	39.36	81.0	-	11.5	9.6
Laurel (*Machilus thunbergii*)	36.28	5.67	8.45	53.06	46.09	83.9	-	12.0	9.9
Glossy privet (*Ligustrum lucidum*)	34.86	9.82	8.96	31.02	33.80	56.2	-	8.0	6.6
Willow (*Salix spp.)*	31.16	12.86	14.11	34.09	34.05	96.5	-	13.7	11.4
All browses	35.79	8.52	10.26	39.85	34.99	619.0	-	88.2	73.0
Apple	12.71	9.16	2.51	9.48	8.42	16.4	100.0	2.3	1.9
Carrot	5.54	18.82	10.38	19.74	21.51	6.8	99.3	1.0	0.8
Green bean	4.83	14.43	22.47	25.24	23.88	5.9	98.7	0.8	0.7
Broccoli	6.11	12.33	32.77	18.10	18.80	6.6	100.0	0.9	0.8
Asparagus	3.73	14.68	30.51	24.99	20.11	3.9	100.0	0.6	0.5
Cucumber	1.86	28.35	26.45	24.43	23.30	3.6	97.6	0.5	0.4
All fruits/vegetables	5.54	13.98	15.60	17.20	16.47	43.2	99.5	6.2	5.1
Soy bean	37.67	6.49	50.23	14.46	13.24	11.7	100.0	1.7	1.4
Peanuts	89.16	3.66	28.75	39.94	37.04	15.4	75.2	2.2	1.8
Primate L/S biscuit banana (Mazuri)	92.30	8.48	18.46	32.16	23.97	12.8	89.0	1.8	1.5
Others	64.17	6.03	29.87	29.98	25.88	39.9	85.7	5.7	4.7
Ingested diet	27.30	8.71	11.70	37.89	33.33	702.0	-	100.0	82.8

DM, dry matter; ash, crude ash; CP, crude protein; aNDFom, neutral detergent fibre assayed with a heat stable amylase exclude residual ash; ADFom, acid detergent fibre excluded residual ash; DMI, dry matter intake; BW, body weight (kg)

*1 Intake/offered of each feed (DM-basis). Browses were weighed as the whole branch, including leaves and twigs, but nutrient contents were analysed only for leaves. Thus, we could not calculate offered DM amounts of browse.

*2 DM intake of each feed/total DM intake.

*3 BW of the proboscis monkey was 17.3 kg at the beginning of the second week of the experiment.

Table S4. Nutrient contents in the diet and DM intake of an adult male of *Nasalis larvatus* in January 2018 (winter)

	DM (%)	Ash (% DM)	CP (% DM)	aNDFom (% DM)	ADFom (%DM)	DMI (g DM/day)	DMI*1 (%)	DMI*2 (%)	DMI*3 (g DM/kg$^{0.75}$/day)
Bamboo-leaf oak (*Quercus myrsinifolia*)	45.13	10.21	6.63	44.19	32.59	81.7	-	12.47	9.0
Chinquapin (*Castanopsis sieboldii*)	42.09	6.11	8.67	43.38	36.06	56.7	-	8.66	6.3
Laurel (*Machilus thunbergii*)	46.24	10.19	6.63	42.12	36.08	217.4	-	33.20	24.0
Glossy privet (*Ligustrum lucidum*)	29.32	10.55	12.98	24.50	18.40	42.8	-	6.54	4.7
Hibiscus (*Hibiscus spp.)*	22.83	13.74	12.64	12.97	14.67	29.4	-	4.48	3.2
Japanese spindletree (*Euonymus japonicus*)	32.36	12.59	7.84	28.07	24.61	63.7	-	9.73	7.0
All browses	39.10	10.28	7.93	37.51	31.19	491.7		75.1	54.2
Apple	14.62	3.87	2.03	9.92	8.28	23.5	98.3	3.58	2.6
Carrot	7.69	8.76	9.11	13.87	15.40	2.4	25.1	0.37	0.3
Green bean	5.44	12.95	23.88	24.65	24.28	3.9	41.4	0.59	0.4
Broccoli	10.81	9.16	32.11	14.27	13.61	15.7	98.3	2.40	1.7
Asparagus	4.52	12.40	29.97	26.26	19.75	4.4	95.9	0.67	0.5
Cucumber	2.54	16.92	21.39	18.75	19.49	3.0	97.3	0.46	0.3
All fruits/vegetables	8.48	7.78	16.31	14.33	12.95	52.8	79.5	8.1	5.8
Soy bean	39.95	5.91	45.44	17.73	17.25	16.3	99.7	2.48	0.3
Peanuts	95.65	3.69	26.01	44.24	38.20	44.5	63.9	6.80	4.9
Primate L/S biscuit banana (Mazuri)*4	35.55	8.22	20.31	33.18	21.91	49.5	98.8	7.56	5.5
Others	48.69	6.05	26.32	35.37	27.80	110.3	79.0	16.8	12.2
Ingested diet	31.08	9.36	11.71	35.28	29.15	654.8	-	100.0	72.2

DM, dry matter; ash, crude ash; CP, crude protein; aNDFom, neutral detergent fibre assayed with a heat stable amylase exclude residual ash; ADFom, acid detergent fibre excluded residual ash; DMI, dry matter intake; BW, body weight (kg)

*1 Intake/offered of each feed (DM-basis). Browses were weighed as the whole branch, including leaves and twigs, but nutrient contents were analysed only for leaves. Thus, we could not calculate offered DM amounts of browse.

*2 DM intake of each feed/total DM intake.

*3 BW of the proboscis monkey was 18.9 kg.at the beginning of the second week of the experiment.

*4 The pelleted feed was soaked in water before feeding

Table S5. Nutrient contents in the diet and DM intake of an adult male of *Nasalis larvatus* in June 2018 (summer)

	DM (%)	Ash (% DM)	CP (% DM)	aNDFom (% DM)	ADFom (%DM)	DMI (g DM/day)	DMI*1 (%)	DMI*2 (%)	DMI*3 (g DM/kg$^{0.75}$/day)
Yoshino cherry (*Prunus yedoensis Matumura)*	28.74	9.14	10.60	22.09	19.68	121.6	-	18.3	14.0
Laurel (*Machilus thunbergii*)	37.38	4.96	7.46	55.23	44.02	237.4	-	35.7	27.4
Glossy privet (*Ligustrum lucidum*)	28.66	11.90	7.65	31.00	30.49	46.4	-	7.0	5.4
Japanese spindletree (*Euonymus japonicus*)	29.53	8.93	9.61	33.96	23.84	110.9	-	16.7	12.8
All browses	77.73	7.42	8.68	40.68	32.73	516.4		77.7	59.6
Apple	12.39	5.54	1.50	8.29	9.42	20.7	100.0	3.1	2.4
Carrot	5.28	8.58	6.09	14.81	15.68	5.7	88.5	0.9	0.7
Green bean	6.08	8.75	18.07	24.20	24.80	9.0	94.1	1.4	1.0
Broccoli	7.13	12.58	36.77	21.18	21.01	8.7	100.0	1.3	1.0
Asparagus	5.25	10.95	39.55	24.42	18.50	3.9	97.8	0.6	0.5
Cucumber	3.00	17.55	27.53	18.35	19.10	2.9	100.0	0.4	0.3
All fruits/vegetables	7.67	8.76	15.41	15.86	16.08	50.9	97.3	7.7	5.9
Soy bean	40.97	5.86	48.24	17.64	16.13	15.3	100.0	2.3	1.8
Peanuts	88.94	3.84	27.69	33.87	29.95	28.5	52.7	4.3	3.3
Primate L/S biscuit banana (Mazuri)*4	35.55	8.22	20.31	33.18	21.91	53.3	100.0	8.0	6.1
Others	14.60	6.56	26.88	30.93	23.36	97.0	79.2	14.6	11.2
Ingested diet	27.23	7.40	11.85	37.35	30.09	664.3	-	100.0	76.7

DM, dry matter; ash, crude ash; CP, crude protein; aNDFom, neutral detergent fibre assayed with a heat stable amylase exclude residual ash; ADFom, acid detergent fibre excluded residual ash; DMI, dry matter intake; BW, body weight (kg)

*1 Intake/offered of each feed (DM-basis). Browses were weighed as the whole branch, including leaves and twigs, but nutrient contents were analysed only for leaves. Thus, we could not calculate offered DM amounts of browse.

*2 DM intake of each feed/total DM intake.

*3 BW of the proboscis monkey was 17.8 kg at the beginning of the second week of the experiment.

*4 The pelleted feed was soaked in water before feeding

Table S6. Nutrient contents in the diet and DM intake of two adults *Trachypithecus cristatus* in August 2018 (summer)

	DM (%)	Ash (% DM)	CP (% DM)	aNDFom (% DM)	ADFom (%DM)	DMI (g DM/day)	DMI*1 (%)	DMI*2 (%)	DMI*3 (g DM/kg$^{0.75}$/day)
Bamboo-leaf oak (*Quercus myrsinifolia*)	51.48	7.60	9.19	56.62	34.56	16.0	-	2.0	1.7
Apple	14.17	6.12	1.53	5.24	6.24	73.5	95.9	28.1	8.0
Banana peel	8.81	17.68	8.48	30.10	30.67	6.8	94.6	3.2	0.7
Cabbage	8.74	8.62	15.24	14.23	14.50	69.8	98.5	21.7	7.6
All fruits/vegetables	10.76	7.80	8.22	10.55	11.19	150.1	96.9	52.9	16.4
Carrot	10.69	7.59	7.23	10.34	11.27	6.3	97.0	2.0	0.7
Sweet potato	31.66	3.33	2.74	6.88	3.55	101.3	99.2	34.2	11.1
Leaf-Eater Primate Diet – Mini-Biscuit (Mazuri)	91.40	7.90	22.92	27.40	18.90	18.3	100.0	6.9	2.0
Others	31.54	4.21	5.90	10.03	6.16	126.0	99.3	43.1	13.8
Ingested diet	16.00	6.24	7.27	12.85	10.30	292.1	-	100.0	31.9

DM, dry matter; ash, crude ash; CP, crude protein; aNDFom, neutral detergent fibre assayed with a heat stable amylase exclude residual ash; ADFom, acid detergent fibre excluded residual ash; DMI, dry matter intake; BW, body weight (kg)

*1 Intake/offered of each feed (DM-basis). Browses were weighed as the whole branch, including leaves and twigs, but nutrient contents were analysed only for leaves. Thus, we could not calculate offered DM amounts of browse.

*2 DM intake of each feed/total DM intake.

*3 BW of two silver lutungs were 7.6 and 7.6 kg at the beginning of the second week of the experiment.

Table S7. Nutrient contents in the diet and DM intake of two adults of *Trachypithecus cristatus* in February 2019 (winter)

	DM (%)	Ash (% DM)	CP (% DM)	aNDFom (% DM)	ADFom (%DM)	DMI (g DM/day)	DMI*1 (%)	DMI*2 (%)	DMI*3 (g DM/kg$^{0.75}$/day)
Bamboo-leaf oak (*Quercus myrsinifolia*)	50.65	7.64	8.67	56.74	41.46	10.5	-	3.9	1.2
Apple	13.01	4.64	1.94	8.96	7.65	74.9	95.9	28.1	8.5
Banana peel	11.27	15.89	6.77	28.23	28.82	8.5	94.6	3.2	1.0
Cabbage	6.51	10.90	15.32	19.78	22.87	57.7	98.5	21.7	6.6
All fruits/vegetables	4.59	15.76	29.10	30.31	15.41	141.2	96.9	52.9	16.1
Carrot	9.30	9.09	5.74	14.15	15.79	5.4	97.0	2.0	0.6
Sweet potato	28.64	4.09	5.31	8.44	4.19	91.2	99.2	34.2	10.4
Leaf-Eater Primate Diet – Mini-Biscuit (Mazuri)	91.40	7.86	22.92	27.43	18.87	18.3	100.0	6.9	2.1
Others	14.48	9.85	23.46	14.16	16.27	114.9	99.3	43.1	13.1
Ingested diet	13.63	6.60	15.00	12.71	7.93	266.6	-	100.0	30.3

DM, dry matter; ash, crude ash; CP, crude protein; aNDFom, neutral detergent fibre assayed with a heat stable amylase exclude residual ash; ADFom, acid detergent fibre excluded residual ash; DMI, dry matter intake; BW, body weight (kg)

*1 Intake/offered of each feed (DM-basis). Browses were weighed as the whole branch, including leaves and twigs, but nutrient contents were analysed only for leaves. Thus, we could not calculate offered DM amounts of browse.

*2 DM intake of each feed/total DM intake.

*3 BW of two silver lutungs were 6.8 and 7.7 kg at the beginning of the second week of the experiment.