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Research article

## Multi-criteria study on a change to a fruit-free diet in Cebidae and Cercopithecidae

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**Abstract**

Nutrient intake of captive primates does not necessarily reflect that of their wild counterparts. Diets in captivity are often higher in non-structural carbohydrates and lower in fibre, resulting in health issues such as obesity, dental issues, diarrhoea and behavioural problems. The main objective of this study was to establish and monitor a change to a fruit-free diet in five species of primates (*Ateles fusciceps rufiventris*, *Cercopithecus hamlyni*, *Allochrocebus lhoesti*, *Cercopithecus roloway*, *Sapajus xanthosternos*). Nutrition and ethology was monitored, including an assessment of the nutritional composition of diets before, during and after diet change; monitoring of faeces consistency; observation of feeding choices; and the occurrence of aggressive behaviour and vocalisations by scan sampling. The initial diet included cultivated fruits and vegetables and some extras (cereals, animal and vegetable proteins) and was higher in non-structural carbohydrates, in particular sugar, than recommended in husbandry guidelines. After a diet change of four weeks during which fruits were gradually removed, a decrease of mean sugar content by more than half and an increase in fibre was achieved. Improved faeces consistency was observed for spider monkeys *A. f. rufiventris* and Hamlyn monkeys *C. hamlyni* (change in Bristol stool score: 6 to 4 and 7 to 3, respectively). An increase in time spent feeding was observed for capuchins *S. xanthosternos* and Hamlyn monkeys (1.5 to 2 times longer). These findings underline the beneficial effects of changing to fruit-free high-fibre diets in zoo-managed primates.

**Introduction**

As nutrition is one of the five pillars of animal welfare (Mellor et al. 2020), modern zoos aim to provide their animals with a high-quality diet in sufficient quantity. Over the past two decades, zoos have initiated diet changes, moving from feeding animals based on habit and empiricism to feeding based on objective nutritional needs and welfare criteria (Britt et al. 2015; Mellor et al. 2020; Plowman 2013). This transformation of feeding habits is particularly challenging for non-human primates in zoos. Primate diets in zoos are often composed of cultivated fruits. However, cultivated fruits generally have

higher sugar and lower fibre content than wild fruits (Milton 1999; Schwitzer et al. 2009). Negative effects of excessive carbohydrate consumption in captive non-human primates have been identified, e.g., diarrhoea (Cabana 2014), dental issues, low reproductive success, obesity (Streicher 2004) and aggressive behaviour (Britt et al. 2015; Cabana 2014; Plowman 2013).

Shifts to fruit-free diets (without cultivated fruit) in primates have been carried out since the early 2000s in several European zoos. This initiative was first introduced at Paignton Zoo, showing the benefits of removing fruits from the diet of a variety of primate species (*Colobus guereza*, *C. polykomos*, *Cercopithecus*

*diana*, *Papio hamadryas*, *Ateles hybridus* and *Macaca nigra*) (Plowman 2013). Food intake, nutrient composition, individual dental health, body condition, faeces quality and the overall cost of the feeding regimen were improved by the diet change (Plowman 2013). This thus served as a reference for other facilities seeking to change to a more naturalistic diet, and for the development of similar diets in other primate species. For instance, a change to fruit-free diets in lemurs at Paignton Zoo and Newquay Zoo led to a decrease in aggression and self-directed behaviour (Britt et al. 2015). Singapore Nature Reserve replaced eggs, rice, bread and fruit with sprouted legumes, whole grains, vegetables, forage and insects in most of their primates' diets (*Lemur catta*, *Pithecia pithecia*, *Pygathrix nemaeus*, *Trachypithecus auratus*, *Nasalis larvatus*) and also observed a decrease in aggressive behaviour (Sha 2014).

The present study describes the change to a fruit-free diet for five primate species at Mulhouse Zoo. Before the diet change, clinical problems such as obesity and behavioural problems such as gluttony and agonistic behaviour during meals were identified in several primate species. Based on previous studies, the dietary change was expected to correct these problems. However, to the authors' knowledge, the change to a fruit-free diet had not yet been described for some of the species involved in this study (*Sapajus xanthosternos*, *Cercopithecus hamlyni* and *Allochrocebus lhoesti*). The objective was to conduct a multi-criteria study on the effects of the change to a fruit-free diet in five species of frugivorous primates with different levels of folivory in their natural feeding behaviour. In particular, energy and nutrient intake, faeces consistency, body condition scores, time spent eating and behaviours associated with feeding were evaluated before, during and after the diet change. This inclusive study design with nutritional, behavioural and health criteria allows comprehensive understanding of the effects and limitations of these dietary changes.

## Materials and methods

### Subjects and housing

One group each of spider monkeys *Ateles fusciceps rufiventris*, Hamlyn monkeys *Cercopithecus hamlyni*, L'Hoest monkeys *Allochrocebus lhoesti* and Roloway monkeys *Cercopithecus roloway*, and two groups of yellow-breasted capuchins *Sapajus xanthosternos* (Capuchin 1 and 2) were included in the study. The size, structure and main health concerns of each group are summarised in Table 1.

All subjects were housed in the same building. Each group had 24 hr access to an indoor and an outdoor enclosure at all times. All enclosures were visible to the public. The study was conducted between April and June 2021.

### Study design

Data were recorded during three distinct phases. During the first period (I, Initial), the primates were fed the initial diet that included cultivated fruits. During the last period (F, Final), they received the target fruit-free rations. Period C (Diet change) was the change period between the two rations and lasted for 4 weeks.

In each period, behavioural monitoring during feeding was carried out five times for each primate group and body condition scores (BCS) were estimated four times per individual. For 15 days per period, the offered and leftover food items were weighed for each group, and faeces consistency was monitored with 15 faecal samples per group.

### Diet composition

Until the beginning of the study, the primate groups were fed a mixed diet of vegetables (about 30% of the diet as fed), fruits (about 60% of the diet as fed) and some extras such as dog pellets, cereals, cheese, chicken breast, nuts, eggs, flaked maize and insects. After the 4-week diet change, a fruit-free diet was provided to the primates. The fruit-free diet consisted mainly of vegetables, insects, pellets, and various nuts accounted for an average of 33.4% of the daily energy intake (Table 2). Due to their more folivorous nature, additional leaves were also included on a daily basis for Hamlyn monkeys and on alternate days for Colombian black spider monkeys.

To facilitate the work of zookeepers and due to seasonal variation in vegetable supply, vegetables were categorised into four groups: green leaves, starches, high sugar content and medium sugar content, based on the criteria detailed in Table 3. Criteria were decided internally using composition tables from the French Agency for Food, Environmental and Occupational Health and Safety (ANSES 2017).

Diet was analysed using Zootrition software. Table 4 compares the initial and fruit-free diets. Non-structural carbohydrates (NSC) were obtained by deduction (%NSC=100-%(protein–lipid–neutral detergent fibre (NDF)–ash)) and represent carbohydrates that are not considered as fibre.

Bananas, chicken breast, flaked maize and dog pellets were removed on the first day of the diet change; then, the rest of the fruits were gradually removed and the quantity of vegetables

**Table 1.** Composition of the primate groups submitted to the diet change

Species	Group size and structure	Main concerns
Capuchin 1	1.2 adult pair and one immature animal	Occasional aggression
Capuchin 2	1.2 adult pair and one immature animal	Frequent aggression, hyperphagia
L'Hoest	1.4 family group including two immature females	Frequent aggression
Roloway	1.2 mother and her two offspring	Frequent aggression, overweight female
Hamlyn	1.2 including one immature male	Frequent loose faeces and diarrhoea
Spider monkey	1.2 two adults and one geriatric female	Overweight females

**Table 2.** Diet ingredients used at Mulhouse Zoo (given as percentage fed) for the original (O) and fruit-free (FF) diets

Species	Capuchin		L'Hoest		Roloway		Hamlyn		Spider monkey	
	O	FF	O	FF	O	FF	O	FF	O	FF
Vegetables	47	92	47	94	66	91	57	93	57	91
Fruits	38	0	47	0	22	0	30	0	36	0
Cereals	3	0	1	0	2	0	3	0	1	0
Eggs	1	0	0.5	0	1	0	1		0.5	0
Dog pellets	3	0	1	0	2	0	3	0	1	0
Primate pellets	1	1	1	1	2	1	2	1	0.7	1
Chicken breast	3	0	1	0	2	0	3	0	1	0
Nuts	3	6	1	4	2	7	3	5	1	7
Cheese	1	0	0.5	0	1	0	2	0	0.3	0
Insects	0	1	0	1	0	1	0	1	0	1
Leaves	0	0	0	0	0	0	0	Twice/week	0	Twice/week

increased in parallel. The amount and variety of fruits distributed at each meal decreased.

#### **Daily food weighing and desiccation coefficient**

Keepers followed a strict feeding plan indicating the amount of daily food to be administered to each of the six groups of primates and its distribution within six meals per day (given at 0830, 0930, 1100, 1400, 1530 and 1700). Daily food and leftovers were weighed using an S30-I-30000 scale (Mettler Toledo, France; B3C; maximum 30 kg; precision  $\pm 2$  g) during 15 days of each period of the study (initial diet (I), diet change (C) and fruit-free diet (F)). Prior to subsequent feeding sessions, all leftovers were removed and enclosures cleaned. As all leftovers lost water because of ambient humidity and temperature, weights were accordingly adjusted using a desiccation factor derived from the estimated moisture lost from similar sets of food placed in a room with the

same abiotic conditions as the enclosures. Food consumption was determined by subtracting the weight of leftovers after application of the desiccation factor from the total amount of food offered.

#### **Body condition scoring**

BCS were evaluated weekly for all study subjects over the entire study period. Scores were assigned according to the *Macaca mulatta* scale (Clingerman and Summers 2012). This scale ranges from 1 to 5. Primates were classified according to their BCS as overweight ( $BCS \geq 4$ ), underweight ( $BCS \leq 2.5$ ) or normal ( $3 \leq BCS \leq 3.5$ ).

#### **Faecal scoring**

For 45 days, all faeces were photographed daily and identified by group. Scores for faeces consistency were attributed from these pictures according to the Bristol scale, ranging from 1 to 7

**Table 3.** Vegetable categories used at Mulhouse Zoo

Category	Criteria	Examples
Green leaves	Fibre/sugar ratio > 2; a lot of leaves	Salad, chicory, cabbage
High sugar content	% Sugar DM > 17	Aubergine, cucumber, tomato
Medium sugar content	% Sugar DM < 17	Broccoli, green bean
Starches	% Starch DM > 30; root vegetables	Potato, sweet potato

(Lewis and Heaton 1997). Scores between 3 and 4 are ideal for the species, whereas scores below 3 are too firm and scores over 4 too soft. Scores 6 and 7 were identified as diarrhoea.

#### Behavioural data collection

All individuals were observed using scan sampling for 20 minutes during the midday meal distribution, for a total of five observations per group and period. Meals were distributed in the indoor enclosure and animals were given free access to the outdoor enclosure during mealtimes. Scan sampling took place in the indoor enclosure. Every minute, food chosen by the observed individual was recorded. 'Other' was used if the individual was in the indoor enclosure but not eating and 'Not visible' if it was outside. All occurrence of aggressions and vocalisations were also counted.

#### Calculations and statistical analyses

Zootrition (E. Dierenfeld, version 2022.02.1) was used for diet analysis. RStudio (RStudio Team, version 2022.07.0) was used for statistical analysis. Kruskal-Wallis tests followed by post hoc Wilcoxon tests with Bonferroni correction were used to compare data among the three phases of the study for each species independently. Statistical significance was set at  $P < 0.05$ . In the text and tables, values given are medians with the first and third quartile values in parentheses, unless otherwise noted.

## Results

#### Food intake

Following the introduction of the fruit-free diet, the quantity of food ingested in terms of dry matter increased significantly for all groups except for L'Hoest and Roloway monkeys (Figure 1).

#### Energy and nutrient intakes

For all groups except Roloway monkeys and Capuchin 2, energy intake during the midday and evening meals decreased when switching to the fruit-free diet. The proportion of consumed versus distributed energy decreased significantly for spider monkeys, Capuchin 1 and Hamlyn monkeys (Table 5). Although the total amount of energy consumed by the L'Hoest monkey group decreased, the proportion of distributed energy that was consumed increased (81.9% to 94%). The origin of energy consumed changed significantly for all groups of primates with an important decrease in digestible carbohydrate and an increase in fat and proteins.

#### Dietary preferences

To estimate the dietary preference of each group, the first food consumed by each individual during each observed meal was recorded (Figure 2).

#### Feeding duration

Feeding duration, measured during the first 20 minutes of the midday meal, increased significantly in capuchins, which is consistent with the increase in food intake (Figure 3). The largest increase in feeding time was observed for the L'Hoest monkeys, which more than doubled their median feeding duration. There was a significant increase in feeding duration between the initial and diet change phases for Hamlyn monkeys. However, during observations in the final phase, the Hamlyn monkeys did not eat at several mealtimes, creating a bias in the obtained results.

#### Body condition scoring

The BCS of 12 of the 20 individuals remained unchanged (Table 6). All changes that occurred were of a magnitude of 0.5 BCS

**Table 4.** Daily amounts (per group) and nutrient composition of the original (O) and fruit-free (FF) diets for all primate groups in the study based on the food sheets distributed to the animal teams

Species	Capuchin		L'Hoest		Roloway		Hamlyn		Spider monkey	
	O	FF	O	FF	O	FF	O	FF	O	FF
Amount as fed (g)	2088	2394	5307	4925	2817	2559	2325	2913	4602	5307
Dry matter (g)	894	510	1035	870	957	474	855	486	1764	1035
Metabolisable energy (MJ)	13.3	6.8	13.3	13.0	14.2	7.0	12.7	6.3	27.0	13.3
Energy density (MJ/g DM)	0.015	0.012	0.012	0.015	0.015	0.012	0.015	0.012	0.015	0.012
Protein (% DM)	12.2	22.4	20.6	20.6	13.4	21.4	13.6	22.4	11.7	20.6
Lipid (% DM)	4.9	7.6	8.9	6.6	4.9	7.3	5.3	7.7	5.4	8.9
NDF (% DM)	1.9	9.8	1.9	8.4	2.5	9.6	1.5	7.1	2.8	7.0
Ash (% DM)	3.6	6.9	3.2	6.1	3.4	7.5	3.1	6.2	3.7	6.1
Non-structural carbohydrate (% DM)	77.4	53.8	77.8	55.5	75.2	52.8	82.1	59.2	75.2	58.2

**Table 5.** Median energy and nutrient intake by group monitored during the change from the original diet (O) to a fruit-free diet (FF), with first and third quartile values given in parentheses. \*, \*\* and \*\*\* denote statistically significant differences between the considered period and the initial period (I) with P<0.05, P<0.01 and P<0.001 respectively.

Species	Capuchin 1		Capuchin 2		L'Hoest		Roloway		Hamlyn		Spider monkey	
	O	FF	O	FF	O	FF	O	FF	O	FF	O	FF
ME consumed (% of offered)	95.2	78.7*	96.1	94.4	81.9	94.0*	90.0	90.4	95.2	83.1*	93.9	86.8*
Protein (% of consumed ME)	14.9	24.3***	15.0	24.5***	12.5	23.6***	17.3	23.3***	17.3	25.1***	15.4	24.7***
	[13.6-15.7]	[24.2-24.6]	[13.8-15.6]	[24.3-24.8]	[11.6-13.7]	[23.5-23.7]	[15.8-18.2]	[23.1-23.3]	[16.4-17.5]	[24.9-25.4]	[14.8-15.8]	[24.5-25.2]
Fat (% of consumed ME)	5.3	9.6***	5.2	8.7***	4.9	7.8***	5.2	7.5**	5.3	9.4***	5.1	8.24***
	[5.0-6.92]	[9.4-9.95]	[5.0-6.1]	[8.3-9.2]	[4.8-5.1]	[7.6-8.0]	[5.1-6.9]	[7.3-7.8]	[5.1-6.0]	[8.7-9.6]	[5.0-6.6]	[8.0-8.5]
Digestible carbohydrates (% of consumed ME)	79	66***	80	67***	82	68***	77	69***	77	65***	79	67***
	[77-81]	[65-66]	[78-81]	[66-67]	[80-83]	[68-69]	[76-79]	[69-69]	[76-78]	[65-66]	[77-80]	[66-67]

increments. Three overweight individuals (BCS $\geq$ 4) were among the seven animals in which BCS decreased. A single animal increased its BCS.

#### Faecal scoring

For all groups in the study, mean faecal scores decreased after the diet change, indicating that faeces became firmer (Table 7).

**Table 6.** Mean BCS data (on a scale of 1–5) for Mulhouse Zoo primates during the study. F stands for Female, M for Male, A for Adult and J for Juvenile. The number for capuchins indicates the group

Individual	April 2021	June 2021
Capuchin 1 (M,A,1)	3.5	3.0
Capuchin 2 (F,A,1)	3.0	3.0
Capuchin 3 (F,J,1)	3.0	3.0
Capuchin 4 (M,A,2)	3.5	3.0
Capuchin 5 (F,A,2)	3.0	3.0
Capuchin 6 (F,J,2)	2.5	2.5
L'Hoest 1 (M,A)	3.0	3.0
L'Hoest 2 (F,A)	3.5	3.0
L'Hoest 3 (F,A)	3.0	3.0
L'Hoest 4 (F,J)	3.0	3.0
L'Hoest 5 (F,J)	3.0	2.5
Roloway 1 (M,A)	3.0	3.0
Roloway 2 (F,A)	3.5	3.5
Roloway 3 (F,A)	4.5	4.0
Hamlyn 1 (M,A)	2.5	3.0
Hamlyn 2 (F,A)	3.5	3.5
Hamlyn 3 (F,A)	3.5	3.5
Spider monkey 1 (F,A)	4.0	3.5
Spider monkey 2 (F,A)	4.0	3.5
Spider monkey 3 (M,A)	3.5	3.5

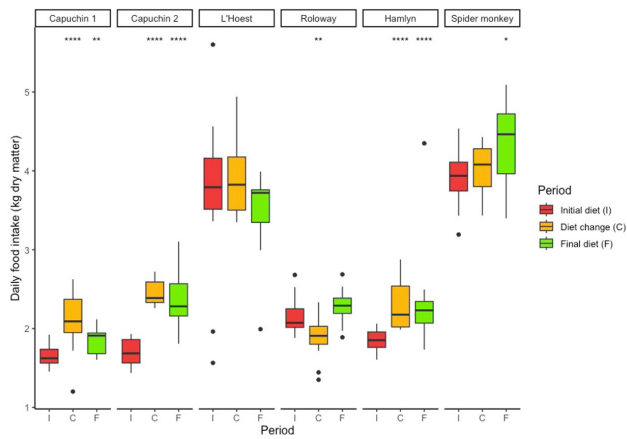
#### Vocalisations

Vocalisations during feeding times were one of the major concerns reported by the zookeepers. For all groups except Capuchin 2, there was a significant decrease in vocalisations during the 20 minutes following midday food distribution (Figure 4). Aggressive behaviour was observed too rarely during feeding at any of the feeding periods to allow a reasonable comparison.

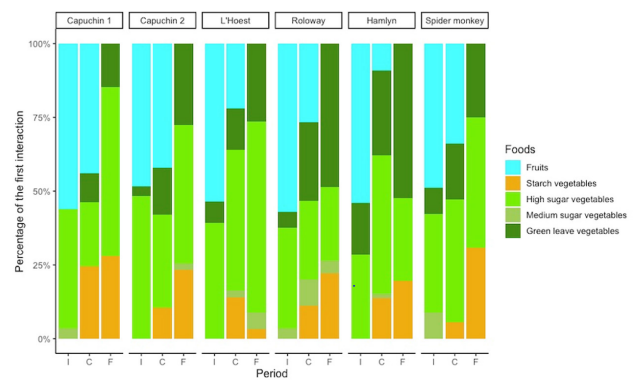
#### Discussion

The five primate species studied here are known as frugivorous (Colyn and Rahm 1987; Curtin 2002; Dew 2005; Hansell et al. 2020; Kaplin and Moermond 2000). 'Fruits' in the common sense used for human food differ in many ways from the fruits that these primates can find in their natural environment. For the same amount of energy, wild fruits provide more protein, fat, fibre and vitamin C and less easily digestible carbohydrates (Schwitzer et al. 2009). The composition of easily digestible carbohydrates is also different: sugars of wild fruits are mainly composed of fructose and glucose while in cultivated fruits glucose is replaced by sucrose (reviewed in Milton 1999). Vegetables (e.g., aubergine, cucumber, tomato, broccoli, green beans) have a composition much closer to that of wild fruits in terms of macronutrients, vitamins and sugar (ANSES 2017).

During the initial observation period (I), the high attractiveness of the fruits was observed for all species (Figure 2). Even after a large decrease in fruit quantity in the proposed diet during the diet change period, fruits remained one of the first food items eaten by the primates. One hypothesis to explain this preference for cultivated fruits is their high sucrose content. Indeed, in humans, sucrose has a much higher sweetening power than glucose (which is more present in wild fruits) (MacDonald 1988 cited in Milton 1999). The second hypothesis is the higher energy density of fruits, especially because of their lower fibre content. Predominance of starch-rich vegetables as the first choice of food during meals after the diet change would confirm the second hypothesis. However, after the removal of fruits, food preferences seem to be much more diversified, with differences between primate species. Only vegetables with a medium sugar content remain almost absent from the first choices of the monkeys after the diet change. A longer observation of the food preferences within a fruit-free diet is needed to confirm this diversification.



**Figure 1.** Daily food intake as dry matter by each group of primates on each diet. \*, \*\*, \*\*\* and \*\*\*\* denote statistically significant differences between the considered period and the initial period (I) with  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  and  $P < 0.0001$ , respectively



**Figure 2.** First food consumed per individual of each group following the distribution of the midday meal. I = initial period; C = diet change period; F = fruit-free diet

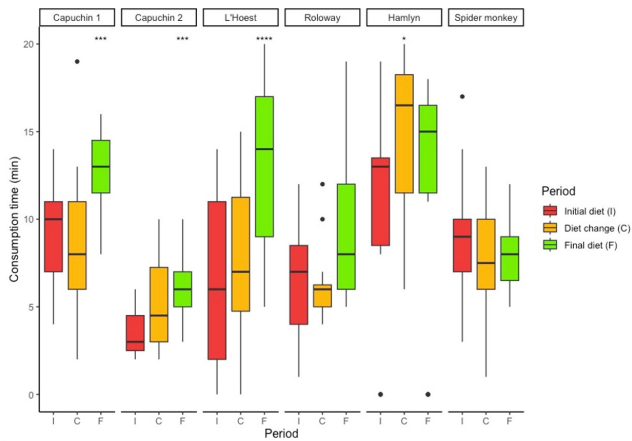
Even though aggression could not be reasonably scored based on aggressive interactions, according to the zookeepers, mealtimes had less conflict after implementation of the fruit-free diet. This observation is substantiated by the decrease in vocalisations in almost all groups and is consistent with observations of lemurs (Britt et al. 2015). Conflicts associated with a high sugar diet have been described in the literature, notably in human medicine, and an increasing amount of research on animal models seems to link sugar to drug-like effects that may explain the problematic behaviours associated with sugary foods (Avena et al. 2008; DiNicolantonio et al. 2018; Zorrilla and Koob 2019). The diet change did not appear to have a significant effect on the number of vocalisations in the Capuchin 2 group, whereas it did in the Capuchin 1 group despite similar housing conditions and group composition. Capuchin 2 is a social group with more tension and a smaller inside enclosure. Moreover, the individuals in Capuchin 2 spent more time eating indoors together after the diet change, whereas they ate outside during the first phase of the study. These

points suggest that although tensions remained in this group, the dietary change resulted in less conflict-associated access to food. Longer-term monitoring of the effect of diet change on Capuchin 2, as well as ethological observations in the outside enclosure, would be useful to determine the actual effect of removing fruits from the diet on the social behaviour of the animals.

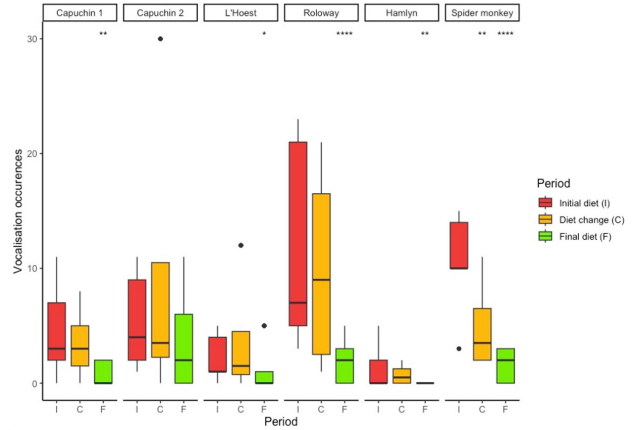
Beyond a modification of sugar intake, the removal of fruits and their replacement by vegetables has other consequences on the ration that may have had repercussions on the animals. By increasing the amount of fibre and therefore decreasing the energy density of the ration, an increase in satiety leading to a decrease in the amount of energy ingested was expected. A decrease was observed in Capuchin 1, L'Hoest and spider monkey groups. In the Capuchin 1 and spider monkey groups, this was combined with a decrease in the proportion of energy consumed in relation to that distributed, confirming a higher satiety. In addition, the decrease in energy intake allowed a reduction in and normalisation of the BCS of the two overweight black spider

**Table 7.** Median (minimum–maximum) faecal scores (on a range from 1 (very firm) to 7 (liquid diarrhoea), Bristol scale) for primate groups in Mulhouse Zoo during the study

Period	Capuchin 1	Capuchin 2	L'Hoest	Roloway	Hamlyn	Spider monkey
April 2021	3.7 (3.3-4.2)	3.7 (3.2-4.2)	4.3 (3.7-4.8)	4.8 (4.0-5.7)	5.0 (4.4-5.2)	5.6 (5.0-6.1)
June 2021	3.3 (3.0-3.7)	3.0 (3.0-3.0)	3.7 (3.5-4.0)	4.6 (4.0-5.3)	3.9 (3.5-4.2)	4.2 (3.8-4.6)



**Figure 3.** Duration of food consumption per individual of each group in the first 20 minutes following distribution of the midday meal, by period and diet. \*, \*\*, \*\*\* and \*\*\*\* denote statistically significant differences between the considered period and the initial period (I) with  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  and  $P < 0.0001$ , respectively



**Figure 4.** Number of vocalisations in the 20 minutes following the distribution of the midday meal. \*, \*\*, \*\*\* and \*\*\*\* denote statistically significant differences between the considered period and the initial period (I) with  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  and  $P < 0.0001$ , respectively.

monkeys. However, satiety does not seem to have been reached in L'Hoest monkeys. Indeed, although feeding time increased and vocalisations decreased, the proportion of energy consumed as a proportion of that offered increased. This lack of satiety might not be due to the fruit-free diet, but rather to a concomitant change in the amount distributed to this species. The study revealed that the amount of food originally distributed to the L'Hoest monkeys had been calculated on an erroneous basis that counted the 6-month-old female still nursing as an adult. This overestimation resulted in the wastage of almost 20% of the quantity distributed in the initial diet.

The fruit-free diet had positive effects on stool quality, especially in Hamlyn and spider monkeys. In these two species, stools became significantly firmer after dietary change. While faeces of spider monkeys were often soft or diarrhoeic during the initial observation phase, stools of the whole group became firmer with the diet change, and a faecal score of 4 was reached at the end of the study, compared with an ideal score of 3 (Lewis and Heaton 1997). Change to a fruit-free diet had a similar effect on faeces consistency in Hamlyn monkeys. Before the diet change, the male of the group was prone to frequent watery diarrhoea of unknown aetiology. Repeated bacteriological and parasitological testing always showed negative results and this phenomenon only responded to the short-term action of Smecta ND (diosmectite, Ipsen Consumer Healthcare, Boulogne Billancourt, France) before the diet change. However, as soon as fibre intake was increased, an effect was noticed on the stools of this group: faeces reached a firm and ideal consistency during the final phase. Softer stools were still observed occasionally, often associated with sugar-rich vegetables such as tomatoes or beetroot, or increased visitor density. Indeed, these episodes of softer faeces or diarrhoea may also be associated with the administration of unusual feeding items such as chips, candy or chocolate by the visitors despite prohibition signs placed in front of the enclosure. It should be noted that the lack of effect of the diet change on faeces consistency in the Roloway group can be explained by the fact that the male

Roloway monkey was on antibiotics (amoxicillin-clavulanic acid, 20 mg/kg q 12 h PO) during the study and diarrhoea is one of the main potential side effects of this treatment.

Before the diet change, there was some concern among the zookeepers at Mulhouse Zoo regarding acceptance of the new diet, with fruit being reported as the preferred ingredient, and regarding whether the primates would become more aggressive, even temporarily, during the gradual withdrawal of fruit. Doubts were also raised about whether expectations in terms of improved health and behaviour were too high. Despite negative staff perceptions of this diet change, the animals became slimmer and intra-group tension reduced. The change in rations has improved the acceptance of new vegetables by the primates. The staff noted that leafy vegetables were rarely sorted and new foods such as mushrooms that were assumed by the staff to be unpalatable are now welcomed by the animals. These results are consistent with those obtained in a survey of zookeepers who noted that monkeys were motivated by their diet whether the rations were with or without cultivated fruit (Hammerton et al. 2019). According to staff feedback, categorisation made the preparation of the ration easier, although preparation time was longer than for the original diet.

While this study provides significant results, there are many limitations that should be considered in future work. One of the main biases is the strong decrease in L'Hoest monkeys' feeding quantity at the same time as the dietary change. More generally, the study was conducted between April and July 2021 and does not consider the effects of seasonality such as perceived variation in food quality or variation in food intake due to weather and temperature. Additional parameters would be interesting to study. For instance, BCS is a subjective measure that should be studied over a long period of time, which may explain the lack of change found in this study. Ideally, a protocol of weighing animals prior to diet change should be implemented to allow more accurate monitoring (Plowman 2013). However, the animals involved in this study were not trained for weighing. Had blood

samples been taken from the primates before and after the dietary change, health status and blood triglyceride values could have been monitored. Finally, a multi-year study including animals from several zoos should be considered to better understand the advantages and limitations of fruit-free rations for primates.

#### Conclusion

The present study highlights findings regarding the switch to a fruit-free diet in five different species of primates based on various criteria: food intake, energy consumed, body condition score, faecal score, feeding duration and vocalisations. Diet change to a fruit-free diet had beneficial effects on the health and behaviour of the primates from Mulhouse Zoo included in the study. Its implementation allowed a significant decrease in the amount of available energy and non-structural carbohydrates administered to the animals, and an increase in the proportion of neutral detergent fibre delivered. Moreover, groups seemed to ingest less energy after the dietary change. These changes in diet composition and ingestion had clear positive impacts on the behaviour (fewer vocalisations) of the frugivorous species and on the faecal scores of the folivores.

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