

Trialing nutrient recommendations for slow lorises (*Nycticebus* spp.) based on wild feeding ecology

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Summary

Slow loris (*Nycticebus* spp.) captive diets have been based on routine and anecdotes rather than scientific fact. The growing body of evidence contradicts the high fruit diet supported by such anecdotes. Non-human primate nutrient requirements are grouped into new (based on the common marmoset *Callithrix jacchus*) or old world (based on rhesus macaques *Macaca mulatta*) primates. Slow lorises are known to suffer from many health ailments in captivity such as dental disease, obesity, wasting and kidney issues all of which have been linked to diet. This study aims to estimate nutrient intake from free ranging slow lorises and to determine if this intake can be used as nutrient recommendations. We collected data of nutrient intake, food passage rate and digestibility of captive slow lorises on three diet treatments 1: current captive type diet which is mostly fruits, 2: wild type diet made only of food items from their natural diet, 3: new diet made to reflect wild slow loris nutrient intake. In order to validate our nutrient recommendations, diets 2 and 3 would have to be significantly different to Diet 1 in terms of nutrients, but not different from each other. Captive diets were significantly higher in soluble carbohydrates and lower in minerals and fibre fractions than both diets 2 and 3. Diets 2 and 3 led to a significantly increased food passage time and to more effective fibre and calcium digestion compared to Diet 1. We also observed obese individuals lost weight and underweight individuals gained weight. Our nutrient recommendations have been validated by our trials, and new or old world monkey nutrient

recommendations are not consistent with our results. Diets should be high in protein and fibre and low in soluble carbohydrates and fats.

Keywords: diet, primate, mean retention time, digestibility, intake, nutrition

Introduction

Feeding wild animals in captivity is a challenge due to their estimated nutritional needs being based on model species (O'Sullivan et al., 2013). Nutrient recommendations exist largely for domestic or laboratory species because this area of research is well funded and has an extensive sample size which is not the case for exotic animals. Non-human primates were prescribed one of two nutritional models, old world monkey (OWM) which based its nutrient requirements on the rhesus macaque (*Macaca mulatta*) or the new world monkey (NWM) which is based on those for the common marmoset (*Callithrix jacchus*) (NRC, 2003). Both of these artificial groups involve many species which are distantly related and have very different physiologies, behaviour and ecology. The OWM group in particular has a large variety of primate taxa which have been shown to not fit the OWM model, such as Lemuridae (Junge et al., 2009; Donadeo et al., 2016; Dierenfeld and McCann, 1999), Colobinae (Nijboer and Dierenfeld, 1996), Hominidae (Crissey et al., 1999; Hoffer 2016; Less et al., 2014) and Lorisidae (Williams et al., 2015). There is evidence that the majority of taxa require their own unique nutritional requirements and using a "one model approach" may not be appropriate (NRC, 2003). Strepsirrhines are particularly affected, especially *Nycticebus* spp. due to their specific exudativorous feeding ecology and abundance of health issues observed in captivity (Cabana and Nekaris 2015). *Nycticebus* spp. have a morphology and physiology adapted to consume and exploit plant gums as a staple food source (Nekaris 2014). Their dentition is specialised to incisiform canines to form a tooth comb as well as procumbent tusk like pre-molars (Kubota and Iwamoto, 1967). They have a long narrow tongue able to lap up gum that has not yet dried or nectar (Coimbra-Filho and Mittermeier 1978). Their gastrointestinal tract (GIT) is also described to be specialised, with a wide large intestine and a voluminous caecum, suggesting their capability for fermenting plant structural carbohydrates (Stevens and Hume 1995). These adaptations are convergent with the gum feeding marmosets (*Cebuella*, *Callithrix*

spp.) (Smith 2010). Field research also confirms that gum is available all year long and is used as a staple food item for the pygmy slow loris, which spends on average 30% of its foraging time on gum (*N. pygmaeus*: Starr and Nekaris 2013), 66% of foraging time for the greater slow loris (*N. coucang*: Wiens, 2002), 96% of foraging time for the Bengal slow loris: (*N. bengalensis*: Das et al. 2014) and 52% of intake for the Javan slow loris (*N. javanicus*: Cabana et al., in press; Rode-Margono et al., 2014). These primates are kept in captivity as illegal pets, popular within Japan, Russia, Indonesia, Czech Republic and the United States (Nekaris and Jaffe 2007) and in zoos worldwide as well as Asian rescue and rehabilitation centres. In spite of the evidence for their exudativorous feeding ecology, this has not been represented in their captive husbandry.

Nycticebus primates are found in 79 accredited zoos worldwide (Zoological Inventory Management System, Species360, USA), most of which are being fed a diet far removed from their wild diet which does not cater to their morphology or physiology (Fitch-Snyder et al., 2001; Fuller et al., 2013; Cabana and Nekaris., 2015). Zoological institutions worldwide primarily feed these gummivores as frugivores with high amounts of fruits and concentrate feeds, and little if any, gum or insects (Cabana and Nekaris 2015). However, multiple studies have found a link between diet and health issues including kidney, dental, coat and gastrointestinal problems (Debyser, 1995; Fuller et al. 2014). Approximately 60% of captive-held *N. pygmaeus* in European facilities may have dental health issues; and 51% of zoos and rescue centres worldwide holding slow lorises appear to have at least one affected individual (Cabana, 2014; Cabana and Nekaris, 2015). Evaluated diets were high in sugars and starches, and contained low levels of fermentable fibres (acid detergent fibre: ADF; neutral detergent fibre: NDF; gums), factors which have been linked with the occurrence of dental disease (Cabana and Nekaris, 2015). A controlled diet study trialing a naturalistic diet of gum, insects and nectar produced evidence that these primates are able to thrive on naturalistic diets (Cabana and Plowman, 2014). The slow lorises in the study maintained a healthy weight and had an activity budget more similar to wild slow lorises, however no nutrient recommendations were used as developmental guidelines for this diet (Cabana and Plowman, 2014).

There are no published nutrient recommendations for slow lorises. These Asian primates are classified as old world primates (Nekaris and Bearder, 2011) and diet have thus been developed based on generic nutritional requirements for old world primates, using input data from other African and Asian cercopithecine, pongid, and colobine primates. We aimed to determine more adequate nutrient recommendation for slow loris species by using feeding data of wild individuals. We used validation markers (apparent digestibility, food passage rate, and nutrient intake) throughout controlled feeding studies to determine if varying nutrients resulted in quantifiable differences between typical captive diets and diets based on natural feeding history. We aimed to reproduce similar physiologic responses with the new diet as documented in wild individuals. As a proxy for wild animals, captive animals were fed the same food items we observed wild individuals ingesting, in similar proportions.

Materials and Methods

Animals and experimental design

We separated this study into two segments; the first part involved observation of free-ranging slow lorises to calculate average wild nutrient intake(s). The second (experimental) component consisted of controlled feeding trials with diet manipulations based on the observational data, utilizing captive slow lorises and measuring changes in digestive physiology parameters, and forms the basis of this report.

We observed wild, free ranging Javan slow lorises (n=15) for 12 months in an agro-forest environment on the active volcano of Mt. Papandayan, surrounding the village of Cipaganti in West Java, Indonesia. Observation methods and dietary ingredient and intake rate calculations are described elsewhere (Cabana et al., in press).

Captive nutrient intake studies

We conducted captive feeding trials at Cikananga Wildlife Rescue Centre (CWRC), Sukabumi, West Java, Indonesia, where Javan slow lorises (n=15), greater slow lorises (n=15) and Philippine slow lorises (n=4) were housed pending rehabilitation and release. These animals were being fed diets comprising various market fruits, insects and honey. The entire study lasted nine weeks at CWRC as three different trials were fed, each for three weeks. Sample collection and chemical analyses

All food items offered in the original diets at both CWRC as well as all food items observed being ingested in the wild, were sampled for nutritional analyses. Field samples were collected and dried in indirect sunlight for 12 hours, then placed into a plastic zip lock bag with silica gel for a maximum of one week before being sent for analysis (Norconk and Conklin-Brittain, 2004). The samples were processed in the same way as we observed the wild slow lorises processing them so that only the actual food parts ingested by slow lorises were analysed (example being they only ate the mesocarp part of bananas and never the peel, therefore we removed the peel from our samples). All assays within Indonesia (for wild and CWRC samples) were performed at the Lembaga Ilmu Pengetahuan Indonesia (Indonesian Institute of Science; LIPI) in Bogor, West Java, Indonesia. Proximate nutritional analyses were based on the methods described by Norconk and Conklin-Brittain (2004) with the addition of: neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Van Soest, 1996), simple sugars (SNI.01-2892-1992, point 3.1), soluble fibres (AOAC 985.29.2005), calcium (AOAC 985.35/50.1.14.2005), phosphorous (spectrophotometry), magnesium (AOAC 985.35/50.1.14.2005), sodium (AOAC 985.35/20.1.14.2005), copper and iron (SNI 01-2896-1998, Point 5) to ensure comparability. Total estimated water soluble carbohydrates were calculated by 100-ash-crude protein-crude fat-NDF (Hall 2003).

Diet trials

We collected data on the CWRC individuals during three diet interventions, each of which was fed for three weeks. We recorded data on diet ingredients and nutrient intake, food passage rates and apparent digestibility while animals were offered three separate diets. Diet 1 consisted of their original diet, therefore no acclimatisation period was needed. Daily average amounts offered, per individual (regardless of species or weight), of Diet 1 included: katydids (3.4g), peeled oranges (18.3g), peeled banana (44.0g), mealworms (4.9g), crickets (1.3g), peeled rambutans (12.2g), hardboiled chicken egg without shell (2.2g), sapodilla without seeds (17.1g), honey (4.0g), mangosteen (12.9g) and pine beetle larvae (2.1g). We transitioned the slow lorises to Diet 2 over a one week period, and animals were fed the full diet for two weeks before collecting any data. Diet 2 consisted of a wild-type diet, approximately 49 % gum, 20% insects (katydids, sago worms, grasshoppers etc.) , 2% nectar and 29% plant parts by weight as per

Cabana et al. (in press). We phased the animals to Diet 3 over one week, and fed it for a further two weeks before collecting data. Diet 3 comprises a new diet based on the nutrient intake of wild slow lorises, yet composed of food items readily available and affordable to Asian zoos and rescue centres. Diet 3, as offered per individual daily, consisted of mealworms (2.6g), crickets (6.9g), hardboiled chicken egg with shell (1.3g), palm beetle larvae, pupae and adult mix (6.5g), sweet potato (8g), peeled, semi-boiled cassava (6.8g), green beans (9.7g), semi-boiled carrots (2g) and gum arabic (10g made with 2:1 parts powder to water) – essentially replacing fruit with vegetable ingredients plus added gum. We assumed the data gathered during Diet 2 trials as providing “physiological targets” since diets provided the closest approximation for wild slow lorises. We began with the assumption that wild physiological values were optimal and to be used as the golden standard. Every individual was weighed before each diet trial and then again 6 months later.

Intake study

Intake studies were conducted with the captive lorises fed their current diet as baseline data for seven days as per Britt et al. (2015). Weights of food items offered and uneaten food removed from the enclosure were weighed to the nearest 0.1 g. Dessication dishes of food items were also set up and measured at feeding time and at time of clean-up to calculate actual intakes. Their diets were divided into three feedings at: 20:00, 0:00 and 3:00 and insects and produce were offered at every meal, however gum was only given in the first meal.

Passage rate study

The food mean retention time was calculated using a non-digestible marker that was fed immediately prior to intake/digestion trials (Lambert, 2002). Coloured plastic beads were used at first without success. The slow lorises were able to use their sublingual to filter them out and push them out of their mouths. Instead, 0.1g (~1/8 tsp) of glitter was mixed into a quarter of a guava fruit (60 g) which was considered as part of the intake study, per individual. The time of first appearance until last appearance (\pm XX min) was recorded for the glitter and the guava seeds, with four repeats per animal conducted. Transit times (TT) and mean retention times (MRT) were recorded or calculated.

Apparent digestibility study

Faeces were collected every day at clean-up time (1000 hr) and each species' faeces were pooled to ensure adequate quantities for chemical analysis to determine apparent digestibility. We used the passage rate studies to link the correct faeces with the correct daily food intake quantities. We compared the total amount of macronutrients within the faecal samples versus the amounts ingested and used the equations described in Graffam et al. (1998) to calculate apparent digestibility (equation 1). Equation 1: $D_N = \frac{N_i - N_o}{N_i} \times 100$

Where D_N is the apparent digestibility of nutrient N and N_i is the amount in g of nutrient N ingested, N_o is the amount in g of nutrient N in the faeces.

Statistical analyses

All statistical analyses were performed on SPSS version 22 (IBM, USA). We used a Generalised Linear Mixed Model (GLMM) analysis to determine if species or diet had a main effect upon the nutrient intake data. The interaction between species and treatment was also analysed. The data were not normally distributed and assumed a Gamma distribution for all nutrients and analysed with a link identity function. Species and diet were used as fixed factors and cage number was a random factor. Factors which were significant were further analysed in a pairwise post-hoc test with Bonferroni corrections. The TT and MRT data were also not normally distributed, therefore a non-parametric Friedman test was administered to search for significant differences between the three diet treatments. All species were combined within this analysis as values were similar amongst the three species within the three different interventions, and there are no significant physiological differences between the three species (Nekaris 2014). Any significant results from the Friedman ANOVA were then analysed using a post hoc Wilcoxon Signed Rank Test.

Results

Nutrient intake of wild slow lorises

The nutrient content of all food items analysed, including the items ingested by wild slow lorises, are shown in Supplementary Table 1. Each main staple food item was obtained from one or two plant species. Gum was from an Australian acacia tree, *Acacia decurrens*, nectar from *Caliandra* (*Caliandra catothyrsus*), fruits from jackfruit (*Arctocarpus heterophyllus*), and persimmon (*Diospyros kaki*), flowers from eucalyptus (*Eucalyptus spp.*) and leaves from bamboo (*Gigantochloa cf. ater*). The average nutrient intake for free ranging *N. javanicus* is relatively high in protein and fibre fractions and low in fat and sugars (Table 1).

Intake study of three dietary treatments

The average daily nutrient intake of *N. javanicus* (n=15), *N. coucang* (n=15) and *N. menagensis* (n=4) on all three diet interventions (Diet 1=original diet reflecting diets fed in rescue centres and zoos, Diet 2=wild type based on the proportions of food items eaten by wild slow lorises, Diet 3 = new diet based on proposed nutrient intakes) are shown in Table 2. Overall, new diets were highest in protein, fibre and minerals and lower in sugars and fat. The GLMM revealed that diet treatment had a significant effect on all nutrient intakes (crude fat: $\chi^2=601.6$, crude protein: $\chi^2= 519.7$, energy: $\chi^2= 19.686$, soluble fibre: $\chi^2= 117.9$, ADF: $\chi^2= 137.3$, NDF: $\chi^2=78.5$, WSC: $\chi^2= 34.2$, ash: $\chi^2= 104.7$, calcium: $\chi^2= 395.0$, copper: $\chi^2= 92.410$, iron: $\chi^2= 30.4$, magnesium: $\chi^2= 21.73$, phosphorous : $\chi^2= 633.1$, sodium: $\chi^2= 74.5$ and df=2 and $P \leq 0.001$ for all tests). According to post hoc tests, Diets 2 and 3 were more similar to each other (calcium, energy, ADF, NDF, soluble fibre and WSC were not significantly different) when compared to Diets 1 and 2, or Diets 1 and 3 (Table 2). Species was not shown to have a significant effect for any nutrient intake when correcting for body weight. .

Food passage rates

The food passage rate was slow relative to body size and showed little variation between species or individuals. Transit time values did not increase significantly based on the new diets; however MRT values increased significantly ($\chi^2= 49.81$ $P<0.001$) comparing Diet 1 with Diet 2, or Diet 1 to Diet 3 (Table 3). Passage rates of Diets 2 and 3 were not dissimilar. Wilcoxon Signed Rank post hoc tests with

Bonferroni corrections showed that MRT for Diets 1 and 2 ($Z = -5.239$, $P < 0.001$), or Diets 1 and 3 ($Z = -5.213$, $P < 0.001$) were significantly different, while the MRT resulting from Diets 2 or 3 did not differ.

Apparent digestibilities

Due to the small weight of faecal matter excreted by the slow lorises, we had to pool the faecal samples for enough dry matter for digestibility analyses, with only 2 pooled samples achieved for each species.

We only collected enough *N. menagensis* faecal samples for ADF and NDF analyses. The slow loris species were able to digest protein at relatively similar efficiencies when fed all three diets ((76-83%); Table 4), although protein digestibility tended to decrease with increasing dietary fiber from Diet 1 to 2 or 3. Fibre digestibility was also similar amongst species (30-51% for ADF, 52-80% for NDF). Insoluble fiber digestibility slightly increased with the increased ADF values of Diets 2 and 3. Calcium was the only nutrient to display a striking change (~40% to 50-60%) in its digestibility when animals were fed Diets 2 and 3.

Health monitoring of captive slow lorises

The initial BW of the captive slow lorises varied considerably, and some gained weight while others lost throughout the feeding trials. Nonetheless, all individuals ended the experiment at what was considered a healthy weight based on wild averages: *N. pygmaeus*: 360-580 g, *N. coucang*: 635-850 g, *N. menagensis*: 265-800 g, *N. javanicus*: 750 - 1150 g, *N. bengalensis*: 1140-2100 g (Nekaris 2014). Overweight individuals lost on average 77.68 g, SD \pm 56.50 (average 6.21% initial body weight SD \pm 3.31), and underweight individuals gained 85.12 g SD \pm 76.28 (average 5.09% initial body weight SD \pm 2.33) (Figure 1).

Discussion

Diet compositions and nutrient intake

The current captive diet was significantly different than the wild diet of slow lorises, namely higher in soluble carbohydrates and lower in fibre fractions. By using Diet 1 as a proxy for most current diets being fed to slow lorises (Cabana and Nekaris, 2015), slow loris captive diets' lead to significantly different physiological parameters such as food passage time, nutrient intake and digestibility than wild slow

232 lorises. The wild diet of our model species, the Javan slow loris, *N. javanicus*, did not compositionally
 233 resemble the typical captive diet (Diet 1). The wild diet of *N. javanicus* was surprisingly low in fat
 234 (average of 2.37 %) for a diet which contains roughly 20% insects. Fibre fractions were high for such a
 235 small primate (~11 % soluble fibre, 11 % ADF and 19 % NDF), however this was expected due to the
 236 high amounts of plant matter, chitin, and gum within the diets of free-ranging lorises. These values are
 237 low compared to some folivorous primates such as *Hapalemur* spp. which has a diet of 30+ % NDF
 238 (Overdorff and Rasmussen 1995). Insect chitin is also included in the total ADF values although we do
 239 not yet know how important it is to slow loris physiology or metabolism. Simple sugars and water soluble
 240 carbohydrates are very low within the wild-type diet (~3 % and 42% of DM, respectively) which is why
 241 the main goal of Diet 3 was to reduce WSC and increase fibre fractions within the diet. The original
 242 captive slow loris diet (Diet 1) was heavily based on fruit and honey, with some insect or egg protein.
 243 This led to a diet that was very high in WSC (average of 59 %), and average in protein (14 %), fibre
 244 (ADF: 7% NDF: 11% soluble fibre: 3%) and calcium (0.1%), with an inverse Ca:P ratio.
 245 The oldest slow lorises have resided in this rescue centre for five years, and their original reason for being
 246 confiscated (customs seizure, ex-pet, market rescue etc.) has a large effect on their long term health
 247 (Moore 2012). Some have developed stereotypic behaviours and received different diets before arriving at
 248 the rescue centre. This may explain that many of the slow lorises are able to subsist on this diet, some
 249 better than others. Considering this diet may be sub-optimal in comparison with the nutrients proposed as
 250 recommendations (Table 1), they may meet bare minimum requirements of already healthy and non-
 251 breeding adults, made evident by their long term survival at the centre, albeit with some health issues like
 252 dental issues and hypocalcaemia. The protein, fibre fractions and Ca:P ratio were below our
 253 recommended values from the wild diet, with fat and WSC being found in larger concentrations than the
 254 wild. Low fibre and high WSC are symptomatic of captive *Nycticebus* diets, although generally very high
 255 protein content diets are observed, possible leading to other health complications such to renal
 256 pathologies (Cabana and Nekaris, 2015). Our new diet (Diet 3) was significantly closer to the wild diet of
 257 slow lorises in terms of nutrients ingested, and was attained through a diet of gum arabic, insects, eggs

and vegetables. The gum arabic itself was purified into a white powder and did not smell or resemble the wild gum of *Acacia decurrens*. Although its texture was different, the slow lorises still found it palatable and the gum Arabic kept its mineral properties which makes it a suitable food to pair with insects. Insects are high in protein, fat and phosphorous while gum is high in carbohydrates, calcium and other minerals (Appendix 1) which may explain why they are eaten in similar proportions by wild *Nycticebus* (Cabana et al. 2016a). The goal of creating a new captive diet resembling the nutrient intake of the wild diet was accomplished and allows us to use the wild type data as nutrient targets in this study.

Food passage rate validation

The MRT values of all slow lorises fed Diet 3 were similar to the physiologic response of animals fed wild-type Diet 2. Thus our targets were reached for food passage rate, as both TT and MRT values responded in the same manner. This was expected due to the higher ingested fibre fractions of Diets 2 and 3. The minute differences in fibre contents of both diets were also reflected in small, yet detectable differences within the MRT values. Both the Javan and greater slow loris Diet 3 had 2-4 % less overall fibre fractions than their respective Diet 2, which led to reductions in the average MRT values for the species (Table 2). Yet the fibre fractions increased by 3 % for the Bornean slow loris and consequently their MRT increased by 0.60%. The reduction in WSC content had no obvious effect on the MRT, which suggests the anatomy of *Nycticebus* may be responsive to the mechanical presence of fibres within the gum. This also means the microbial communities may not influence MRT, or else they would have increased for all, due to a longer period of time to adjust in a higher fibre substrate. These results are consistent with our hypothesis of fibre being an important part of the diet for slow lorises. With the mere presence of fibre, the MRT values increased to values also seen for colobine monkeys: namely guerezas (*Colobus guereza*: Kay and Davies 1994), the silvered langur (*Trachypithecus cristatus*: Sakaguchi et al. 1991) and the proboscis monkeys (*Nasalis larvatus*: Dierenfeld et al. 1992). Our hypothesis that high fibre (both soluble and insoluble) content diets are important in slow loris digestive physiology was supported by our data. The observed increase in MRT with added dietary fibre is also reported for the exudativorous pygmy and common marmosets (*Cebuella pygmaea* and *Callithrix jacchus*) (Power 1991;

Power and Oftedal 1996; Power 2010). This effect was not seen in related frugivorous/insectivorous callitrichids, who do not need to rely on gum for nutrients/energy and therefore never evolved to exploit this food item fully. The larger MRT values for the slow lorises on Diets 2 and 3 may allow the digestion and assimilation of not only fibre, but other nutrients as well.

Apparent digestibility validation

The more naturalistic diets (Diets 2 and 3) allowed all three species to digest and assimilate an overall larger amount of each nutrient measured. The amount of protein in Diets 2 and 3 was almost double the amount of protein found in Diet 1, however the apparent digestibility of protein remained similar and only decreased slightly when animals were fed Diet 3. *Nycticebus* has the capacity to digest and assimilate protein and in our study, where the majority of protein was from insects, the efficiency seemed to decline above 23% of DM, which is our recommended amount, even if in captivity their minimum requirements are surely lower (Flurer et al. 1987). Apparent digestibility of fibre fractions increased by 5-10% for ADF and 9-19 % for NDF, which are values similar to the folivorous sifakas (Schmidt et al. 2005a). The actual proportion of ADF and NDF in the diet increased by 2-3.5 % and 10-15% for NDF with the diet revisions, meaning the slow lorises were able to become more efficient in digesting fibre when there was more fibre in the diet. The larger MRT values associated with Diets 2 and 3 may have increased the opportunity for the slow lorises to ferment and digest the cellulose in their large intestine and caecum. The fibre in the diet could possibly be further increased, at least until a maximum digestibility is achieved as shown by Schmidt et al. (2005b) when orangutan NDF digestion began to drop when NDF increased >53% of dietary DM.

Diets higher in fibre and lower in WSC are also conducive to a change of gut microbial communities, to species with higher cellulolytic abilities (Amato 2016). We posit that the gut responded to the increased fibre fractions, which lead to the gut microbes having more time to act upon a larger amount of fermentable substrate. This selection pressure caused a shift in the microbe communities, possibly affecting an increase in fermenting species, further increasing fermentation capabilities. This reflects the wild feeding ecology of the slow lorises which is largely based on gum (soluble fibre) as an energy

source. Lastly, the calcium uptake from the diet increased by up to 50% with increased dietary fiber. It is possible the higher MRT values also helped the assimilation of calcium, either through normal active uptake processes, or perhaps also through more chances for chitonolytic bacteria to hydrolyze chitin and release calcium chemically bound in the insect exoskeleton, allowing it to be assimilated. The results from Table 4 must be interpreted conservatively due to the pooling of faecal samples and small sample size, however this information is still useful when used to compare between diets. Diets 2 and 3 both led to similar amounts of nutrients being digested when compared to Diet 1.

Health impacts validation

The largest effect (or impact) on health was related to the increase in fibre fractions, and reduced water soluble carbohydrates (sugars and starches) from Diet 1 compared to Diet 2 and 3 (which were similar nutrient wise). Besides the observed link between increased fibre and satiety leading to a reduction in abnormal behaviours (Remis and Dierenfeld 2004; Less et al. 2014); the addition of fibres may help modulate the glucose tolerance of the slow lorises, buffering hunger and reducing food intake rates (Jenkins et al. 2000). Anecdotally, the overweight animals were more dominant over food resources, displacing the smaller, thinner individuals. This may be why we observed the overweight individuals losing weight and reducing their dominance over food, which then allowed underweight subordinate individuals to ingest more food. We observed a tendency that food was less guarded once fruit was removed and less displacement occurred in social groups. In other hindgut fermenters, the addition of fibre to standard diets reduced the overall rate of starch digestion (Vervuert et al. 2009). Perhaps the inclusion of root vegetables, typically higher in soluble carbohydrates than other vegetable types, to a diet high in gum may not lead to the harmful effects of WSC on gut microbial communities reported in some dietary studies (Amato 2016). Stool quality should also be improved on higher fibre diets (Sunvold et al. 1995). Although we did not quantify these data, we did notice more solid faeces from animals fed Diets 2 and 3 when compared to Diet 1, considering scraping was required to gather faecal samples for Diet 1 on more than one occasion. Both the black and white colobus (*C. guereza*) and the spectacled leaf monkey (*T. obscurus*) also benefited from better formed faeces under a higher fibre diet (Nijboer et al. 2006), as

do apes (Remis and Dierenfeld, 2004). This may also reflect a healthier overall gut function and more cohesive and responsive gut microbial community (Clayton et al. 2016). The lowered WSC content of Diets 2 and 3 would potentially promote a luminal pH more consistent with one of optimal short chain fatty acid production (Gomez et al. 2016b). Coupled with the increased amount of fibre substrate particles, this should shift the population of gut microbes to one mostly adapted for structural carbohydrate fermentation (Clayton et al. 2016). Predominantly cellulolytic gut microbial communities have been linked with enhanced protection from pathogenic microbes, modulating the immune function, and optimising energy conversion and harvesting efficiencies (Gomez et al. 2015a).

Captive feeding recommendations

The results from our three quantified variables: nutrient intake, food passage, and digestibility were all consistent with Diet 3 promoting physiological values for *Nycticebus* spp. more consistent with free-ranging animals than results obtained on the typical captive Diet 1. The data gathered here also help us to determine that *Nycticebus* are adapted to utilize the nutrients and energy within fermentable fibres, which can greatly benefit both oral and gastrointestinal health in this group of species. If dietary nutrient recommendations of Table 1 cannot be duplicated, at the very least every effort to decrease dietary WSC and increase fibre fractions should be made in the feeding management of captive lorises. This is easily achievable by removing fruits and reducing the concentrate feeds to a more appropriate amount and focusing on vegetables and gum Arabic instead. Positive differences were observed at the CWRC but also in other zoos which have also reduced WSC and increased overall fibre such as in gorillas (Lukas et al. 2014), lemurs (Britt et al. 2015), pygmy slow lorises (Cabana and Plowman, 2014) and slender lorises (Williams et al. 2015).

Neither the nutrient recommendations for old nor new world monkeys were a close match for the slow lorises (NRC, 2003). The higher protein content was more similar to NWM, however calcium was closer to OWM recommendations. We expected the similarity in feeding ecology between the slow lorises and marmosets to result in similar nutrient requirements, but we have shown that wild and captive slow lorises thrive on nutrients very different than recommended for NWP.

Conclusion

The diet created with the nutrient framework of wild slow loris intake led to similar physiological responses as we assume those of a free ranging wild slow loris to be. The nutrient intakes were more similar to each other, notably higher in fibre and lower in soluble carbohydrates, when compared to the original captive diet. This led to longer food mean retention time and higher fermentation capacity for fibre fractions and calcium. The new captive diet emulates wild feeding responses and has led to the medium term stabilisation of slow loris weights and reduction in health issues. Our nutrient recommendations have been validated using the techniques above. Our results indicate the importance of researching diet/nutrient recommendations for a variety of species which do not fit the typical New or Old world monkey model. Future studies should focus on dental health issue progression on lower sugar diets.

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Table 1: Average daily nutrient intake of wild Javan slow lorises (*N. javanicus*; n=15) with a diet consisting mainly of gum, insects and nectar. These nutrient values also reflect the proposed dietary nutrient recommendations for *Nycticebus spp.*

Nutrient	Concentration (DM basis)	Nutrient	Concentration (DM basis)
Energy (Kcal/g)	3.15 (± 0.48)	Ca:P Ratio	2.8:1
Crude Protein (%)	23.50 (± 8.35)	Cu (mg/kg)	11.22 (± 1.4)
Crude Fat (%)	2.37 (± 1.04)	Fe (mg/kg)	69.16 (± 9.34)
Soluble Fiber (%)	10.67 (± 7.86)	Mg (%)	0.37 (± 0.09)
ADF (%)	10.95 (± 7.02)	Na (%)	0.38 (± 0.10)
NDF (%)	19.14 (± 5.5)	Vit A (IU A/g)	2.06 (± 0.56)
Ash (%)	2.24 ($\pm .94$)	Vit D (IU A/g)	0.53* (± 0.23)
Ca (%)	0.45 (± 0.23)	Vit E (mg/kg)	0.97* (± 0.36)
P (%)	0.16 (± 0.11)	Soluble Sugars (%)	3.33 (± 1.52)

*Data represented by less than 80 % of the ingredients

Table 2: Average nutrient intake of *N. Javanicus* ($n=15$), *N. coucang* ($n=15$) and *N. menagensis* ($n=4$) at CWRC under three different dietary regimes., with Diet 1 being the original captive diet high in fruit, Diet 2 a naturalistic diet made of food items eaten in the wild by *N. javanicus* such as insects and gum and Diet 3, a diet of locally found food items such as vegetables, insects and gum with \pm standard deviation.

Nutrient	Diet 1°	Diet 2°	Diet 3
Ash (%) *§¶	3.80 \pm 0.38	3.87 \pm 2.04	5.50 \pm 0.11
Crude Fat (%)§¶	5.77 \pm 0.72	10.63 \pm 1.88	12.98 \pm 0.99
Crude Protein (%)§**	13.57 \pm 0.82	22.65 \pm 3.35	24.74 \pm 2.00
WSC (%) *†	60.22 \pm 2.23	37.93 \pm 8.04	34.71 \pm 2.37
Soluble Fibre (%)‡§	2.76 \pm 0.25	5.04 \pm 1.33	4.34 \pm 0.30
ADF (%)†‡	5.40 \pm 1.91	7.09 \pm 1.93	6.29 \pm 1.41
NDF (%)‡§	6.64 \pm 0.57	19.97 \pm 3.78	18.75 \pm 0.48
Calcium (%)‡§	0.23 \pm 0.12	0.34 \pm 0.13	0.54 \pm 0.06
Phosphorous (%)‡§+	0.18 \pm 0.02	0.32 \pm 0.13	0.48 \pm 0.04
Ca:P	1.31 \pm 0.78	2.16 \pm 2.15	1.09 \pm 0.09
Copper (%) *†**	10.04 \pm 0.08	9.35 \pm 3.28	8.80 \pm 0.38
Iron (mg/kg) * **	55.12 \pm 1.59	63.55 \pm 34.52	75.13 \pm 3.00
Magnesium (%) *¶	0.36 \pm 0.02	0.45 \pm 3.41	0.32 \pm 0.05
Sodium (%)‡§**	0.06 \pm 0.01	.19 \pm 0.02	0.20 \pm 0.02
Gross Energy (kcal/g) ‡§	2.99 \pm 0.06	3.63 \pm 0.45	3.28 \pm 0.03

°Data also used in Cabana et al. (In press)

Post Hoc Pairwise statistic results, *: Diet 1 was significantly larger than Diet 2, †: Diet 1 was significantly larger than Diet 3, ‡: Diet 1 was significantly smaller than diet 2, § : Diet 1 was significantly smaller than diet 3, ¶: Diet 2 was significantly larger than Diet 3, **: Diet 2 was significantly smaller than Diet 3. All were significant at $P \leq 0.001$.

WSC = water soluble carbohydrates, ADF = acid detergent fibre, NDF = neutral detergent fibre, Ca:P = calcium to phosphorous ratio.

Table 3: Average food passage rates (TT=transit time and MRT= mean retention time) of *N. Javanicus*, *N. coucang* and *N. menagensis* at CWRC under three different dietary regimes, with Diet 1 the original captive diet, Diet 2 a naturalistic diet and Diet 3 a diet based on derived recommendation values.

	Diet Time	<i>Javanicus</i> n=15	<i>Coucang</i> n = 15	<i>Menagensis</i> n = 4
Transit Time (hours)	Diet 1 (± SD) (range)	25.6 (±2.6) (23.0-31.5)	25.00 (±3.5) (21.5-29.0)	24.2 (±3.2) (21.0-27.5)
	Diet 2 (± SD) (range)	25.6 (±3.4) (24.0 - 29.0)	24.4(±2.1) (24.0 - 26.5)	24.5 (±2.9) (22.5- 27.0)
	Diet 3 (± SD) (range)	25.1 (±4.1) (23.0 - 28.8)	24.7 (±2.7) (22.0 - 28.3)	24.4 (±2.3) (22.0- 27.66)
Mean Retention Time (hours)	Diet 1 (± SD) (range)	33.40(±1.0) (31.0-32.5)	29.70 (±1.5) (27.0-29.5)	32.88(±3.1) (28.0-33.4)
	Diet 2 (± SD) (range)	38.50(±2.0) (34.5-39.0)	38.0(±2.5) (34.0-37.5)	34.13 (±4.1) (30.0-34.8)
	Diet 3 (± SD) (range)	37.50 (±2.0) (34.0-38.3)	37.60 (±2.0) (33.0-37.75)	34.75 (±3.25) (30.0-34.8)

Table 4: Apparent digestibility values for crude protein, acid detergent fibre (ADF) and calcium for *N. javanicus* (*n*=15), *N. coucang* (*n*=15) and *N. menagensis* (*n*=4) at CWRC under three different dietary regimes, with Diet 1 the original captive diet, Diet 2 a naturalistic diet and Diet 3 a diet based on derived recommendation values.

		<i>N. javanicus</i>	<i>N. coucang</i>	<i>N. menagensis</i>
Crude Protein	Diet 1 (%)	82.60	81.80	-
	Diet 2 (%)	80.44	79.28	-
	Diet 3 (%)	78.34	76.05	-
ADF	Diet 1 (%)	38.70	44.60	30.30
	Diet 2 (%)	43.54	49.28	40.46
	Diet 3 (%)	46.40	51.93	42.82
NDF	Diet 1 (%)	58.45	51.69	59.05
	Diet 2 (%)	79.65	71.72	65.61
	Diet 3 (%)	77.35	69.56	68.27
Calcium	Diet 1 (%)	37.60	35.90	-
	Diet 2 (%)	61.03	63.75	-
	Diet 3 (%)	50.07	52.41	-

*It was not possible to collect enough faecal sample material to conduct more than one replicate of the tests for each species. Faeces were collected for the same time period (7 days) for each species .

