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## EFFECT OF DIET ON THE FECES QUALITY IN JAVAN LANGUR (*TRACHYPITHECUS AURATUS AURATUS*)

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**Abstract:** A high intake of easily fermentable carbohydrates and a low intake of fiber material are generally regarded as major factors affecting the health of captive langurs. The effect on fecal consistency of excluding fruits and vegetables from the diet was evaluated in Javan langurs (*Trachypithecus auratus auratus*). Cross-over trials were carried out at Rotterdam Zoo and at the Apenheul Zoo, The Netherlands. During the first and third dietary period, the langurs were fed their usual diet, which contained fruits, vegetables, langur pellets, and browse. During the second period, the vegetables and fruits were excluded from the diet and the diet essentially consisted of pellets and browse. Feces consistency was scored using a fecal score chart developed for langurs. During the second feeding period the feces consistency improved significantly in animals at both zoos. Across all trials, a firmer feces consistency was correlated with an increase in dietary cell wall (measured as neutral detergent fiber) and a decrease in dietary water. It is suggested that the combined decrease in the intake of soluble sugars, the increase of fiber intake, and a lower amount of dietary water in the diet resulted in more solid stools. The results indicate that a dietary neutral detergent fiber content of approximately 46% in dry matter will result in a feces consistency indicative of undisturbed gut function.

**Key words:** *Trachypithecus auratus auratus*, Javan langurs, feeding, nutrition, fecal score, fecal consistency, digestive upset, dietary fiber.

### INTRODUCTION

Javan langurs (*Trachypithecus auratus auratus*) belong to the primate subfamily of Colobinae, which is a part of the family Cercopithecidae. Colobines are folivorous and have a multichambered stomach that is divided into a saccus gastricus, tubus gastricus, and pars pylorica. Food components, including fiber, can be fermented in the stomach of leaf-eating langurs.<sup>2,3</sup>

The natural habitat of the Javan langurs is in Java, and their natural diet consists of young leaves, fruits, and flowers.<sup>7,9</sup> Although recent guidelines recommend the use of high-fiber primate biscuits, green leafy vegetables, and browse<sup>5</sup> for captive langurs, many zoological institutions have not adopted a standardized diet for colobines. In contrast, traditionally, high amounts of starch-containing feeds and vegetables and fruits are provided (Nijboer et

al., unpubl. data).<sup>13</sup> Such a feeding regime may result in gastrointestinal disturbances<sup>7</sup> and often leads to loose stools.<sup>11</sup> Commercial fruits and vegetables have been cultivated to comply with human taste and therefore are high in soluble sugars<sup>8,10</sup> and low in fiber.<sup>15</sup> Indeed, wild fruits and young leaves generally contain less soluble sugars and more neutral detergent fiber (NDF) and acid detergent fiber (ADF) than domesticated fruits.<sup>4</sup>

In captive François langurs (*Trachypithecus francoisi*), it was shown that stool quality was improved when the fiber content of the diet was increased.<sup>12</sup> In that study, the intake of extra fiber was associated with lower consumption of nonstructural carbohydrates. Thus, it could be suggested that a decrease in the intake of nonstructural carbohydrates (NSC), including soluble sugars, has a positive impact on feces quality, possibly by slowing down fermentation in the stomach of colobines.

In the present studies with Javan langurs, we tested the effect of removing fruits and vegetables from the usually fed diet on stool quality.

### MATERIALS AND METHODS

#### Animal housing and conditions

Two groups of group-fed Javan langurs at two different zoological institutions were studied. The estimated body mass for the adult (A) Javan langurs varied from 5 to 7 kg.

A group of five Javan langurs was studied at the Rotterdam Zoo. They comprised a male and two

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**Table 1.** Amounts of feeds supplied (S) and ingested (I) during the various test periods. The amounts are expressed as g fed/animal/day.

| Feeds                   | Diets A        |     |                |     |                |     | Diets B        |     |                |     |                |     |
|-------------------------|----------------|-----|----------------|-----|----------------|-----|----------------|-----|----------------|-----|----------------|-----|
|                         | A <sub>1</sub> |     | A <sub>2</sub> |     | A <sub>3</sub> |     | B <sub>1</sub> |     | B <sub>2</sub> |     | B <sub>3</sub> |     |
|                         | S              | I   | S              | I   | S              | I   | S              | I   | S              | I   | S              | I   |
| Pellet <sup>a</sup>     | 196            | 49  | 332            | 183 | 225            | 142 | 57             | 57  | 93             | 93  | 51             | 51  |
| Browse <sup>b</sup>     | 491            | 177 | 1,036          | 456 | 816            | 294 | 375            | 240 | 592            | 379 | 364            | 233 |
| Vegetables <sup>b</sup> | 815            | 563 | 0              | 0   | 720            | 576 | 903            | 894 | 0              | 0   | 943            | 895 |
| Fruits <sup>b</sup>     | —              | —   | —              | —   | —              | —   | 18             | 18  | 0              | 0   | 29             | 29  |
| Rice balls <sup>b</sup> | 102            | 101 | 61             | 61  | 87             | 86  | —              | —   | —              | —   | —              | —   |
| Other <sup>c</sup>      | —              | —   | —              | —   | —              | —   | 24             | 24  | 45             | 45  | 36             | 36  |

<sup>a</sup> The langur pellet (Hope Farms B.V., Woerden, The Netherlands), fed as a component of diets A, contained, on a 90% dry-matter basis, crude protein, 18.1%; crude fat, 5.8%; nitrogen-free extract (Nfe), 11.1%; neutral detergent fiber (NDF), 48.2%; acid detergent fiber (ADF), 26.4%; acid detergent lignin (ADL), 12.6%; and added vitamins and minerals. The pellet (Mazuri Leafeater Pellet, SDS, Witham, U.K.), fed as component of diet B, contained, on a 90% dry-matter basis, crude protein; 23%; crude fat, 5.0%; NDF, 24.7%; ADF, 16.0%; and added vitamins and minerals (manufacturers' information).

<sup>b</sup> See Materials and Methods for details.

<sup>c</sup> Cheese, nuts, yogurt, eggs, and chicken fillet.

female adults, a female subadult, and a male juvenile. The animals were kept in an inside enclosure measuring 63 m<sup>2</sup>. They had access to an outside island with a surface of 56 m<sup>2</sup>. During the experimental period, the langurs went outside only a few times. Because of the season, there were no palatable plants in the outdoor enclosure. The temperature in the inside enclosure was 18°C. The air was refreshed continuously by a controlled ventilation system. The enclosure was sprinkled daily to keep the humidity high. Artificial light was on from 0700 to 1900 hours. The food was offered in a wooden trough (0.8 × 0.5 × 0.1 m) that was placed on a wooden log, except for the browse that was offered in a ball-shaped rack. During the third week of the study an extra feeding area for the browse was created by a hanging, square-shaped trough (0.4 × 0.4 m). At the Apenheul Zoo, the group of Javan langurs consisted of four adults (one male and three females), four subadult females, and a juvenile male and female. During the study an infant was born. The animals were kept in enclosures of 16 and 10 m<sup>2</sup>, the two enclosures being connected by a tunnel. The langurs also had access to outdoor enclosures of 250 and 12 m<sup>2</sup> in which no plants were available for consumption. The temperature in the inside enclosure ranged between 18 and 20°C. The humidity was about 80%. Artificial light was on from 0830 to 1630 hours. Food was offered in troughs (2.0 × 0.5 × 0.1 m).

**Foods and feeding**

Feeding trials were performed in January and February 2004. Basically, two different diets were fed in the zoos. Diets A were fed at Rotterdam Zoo

(A<sub>1</sub> from 6 to 10 January; A<sub>2</sub> from 23 to 27 January; and A<sub>3</sub> from 2 to 6 February). The traditional diet was fed during the A<sub>1</sub> and A<sub>3</sub> period. During the A<sub>2</sub> period the vegetables were left out during the trial. The traditional diets were fed at Apenheul Zoo during period B<sub>1</sub> and B<sub>3</sub>. During period B<sub>2</sub> the vegetables and fruits were left out. Diet B<sub>1</sub> was fed from 5 to 11 January; B<sub>2</sub> from 20 to 26 January; and B<sub>3</sub> from 10 to 16 February. The time between the observations was used to adapt the animals.

At the Rotterdam Zoo, the amounts of pellets and browse were fed ad libitum during all periods. In the traditional diet, the amount of vegetables was also not restricted. Pellets and rice balls with vitamins were fed at 0800 hours, the browse at 1030 hours, vegetables at 1330 hours, and the remaining amount of browse at 1545 hours during the A<sub>1</sub> and A<sub>3</sub> periods. Details on the pelleted diet are presented in Table 1. The rice balls offered were prepared from cooked white rice (250 g), low-fat yogurt (5 g), fruit syrup (5 g), and 1 g vitamin/mineral mixture (calculated composition in mg/g: calcium 115.4, phosphorus 481, copper 0.9, iron 11.5, selenium 0.02, zinc 91.3, manganese 9.6, vitamin C 9.6, thiamin 0.4, vitamin B<sub>2</sub> 3.8, vitamin B<sub>6</sub> 0.4, and vitamin B<sub>12</sub> 2.9, and in IU/g: vitamin A 961, vitamin D<sub>3</sub> 192, and vitamin E 22.1). The offered vegetables consisted of a mixture of carrots, green peppers, endive, fennel, celery, cucumber, red beets, French beans, and chicory. The browse was a combination of refrigerated, stripped rose (*Rosa* spp.) leaves and defrosted willow leaves (*Salix* spp.). During the A<sub>2</sub> period, the primates received the pellets and rice balls at 0800 hours and the stripped rose and willow leaves at 1100 hours. During the

adaptation period from diet A<sub>2</sub> to diet A<sub>3</sub> it was noted that the intake of pellets and browse increased; in order to ensure an ad libitum regime, it was decided to increase the offered amounts of pellets and browse. In contrast, fewer vegetables were offered in A<sub>3</sub> compared to A<sub>1</sub> to reduce waste; this did not influence vegetable intake.

During periods B<sub>1</sub> and B<sub>3</sub> at the Apenheul Zoo, pellets (Mazuri Leaf Eater Pellets; Mazuri, Witham, U.K.), greens, and sunflower seeds were fed at 0900 hours; browse at 1200 hours; beans and vegetables at 1400 hours; and the remaining browse, cheese, nuts, yogurt, and fruits at 1600 hours. The browse consisted of a mixture of leaves from willow, beech (*Fagaceae* spp.), maple (*Acer* spp.), and blackberry (*Rubus ursinus*), which had been frozen and stored in plastic bags. The mixture of greens and vegetables was similar to the mixture fed at Rotterdam Zoo. The fruits consisted mainly of apples. Sometimes other foods, such as eggs, cheese, chicken fillet, and yogurt, were given. During period B<sub>2</sub> the extra browse was fed throughout the day.

During the trials, the leftovers were collected quantitatively and weighed each morning before feeding. The intake of each diet component was calculated by subtracting the weight of the identified leftovers the following morning from the amount supplied. Because indoor temperature was comparatively low and air humidity was high, no corrections were made for potential water losses in the vegetable leftovers. To analyze the nutrient composition of the total diet, duplicate portions of the diet supplied were made and analyzed. In a similar way, samples of the pooled leftovers from each period were prepared for chemical analysis.

Food offered and consumed per group was divided by the units of adult animals as indicated above—3.5 adults for the Rotterdam group and 7 adults for the Apenheul group—and was presented as the average intake per animal.

### Feces scoring system

The feces scoring system was adapted from a system designed by the Waltham Centre for Pet Nutrition.<sup>20</sup> Grade 1 feces are firm and dry, whereas grade 5 feces comprises diarrhea. Figure 1 shows the langur feces scoring system that was used for this study. Score 2 was considered optimal, because consistency was not too firm or too soft and the shape was comparable to the feces produced by wild langurs.<sup>11</sup> In order to avoid stress among the langurs, feces were scored only once daily. Langurs produce feces when sitting in the artificial trees, which means that dropped fecal boli often break up

into several smaller boli when hitting the ground. As fecal scoring was only performed once daily to minimize stress, all individual boli on the ground were assigned a score. The average value per day represents the mean of all boluses scored on that day. At each facility, feces were always scored by the same person (by MO at Rotterdam and WN at Apenheul).

### Chemical analyses

All feed samples were stored in a freezer. Prior to analysis the samples were dried at 60°C to constant weight, pooled per period, and ground to pass through a 1-mm screen. Samples of the diet offered and diet leftovers were analyzed for dry matter, crude protein (nitrogen  $\times$  6.25), crude fat, ash, and crude fiber, according to the Weende methods.<sup>1</sup> The nitrogen-free extract is defined as dry matter minus ash, crude protein, crude fat, and crude fiber. NDF, ADF, and acid detergent lignin (ADL) were analyzed according to the method described by Van Soest.<sup>18,19</sup> Hemicellulose was calculated as the difference between NDF and ADF. Cellulose is the difference between ADF and ADL. The fraction of NSC is defined as the dry matter minus ash, crude protein, crude fat, and NDF.

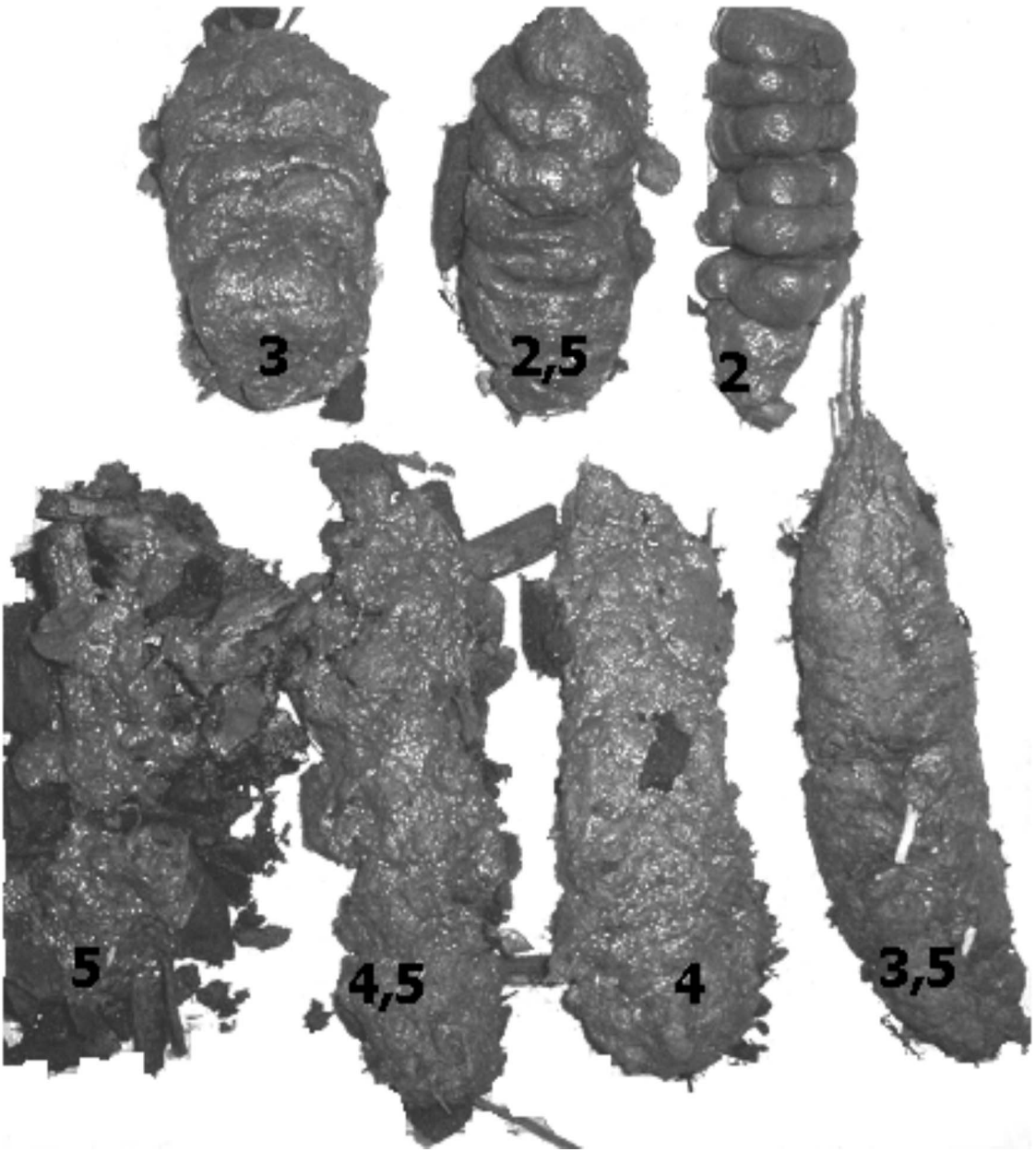
### Statistics

As the fecal scores at both facilities were not normally distributed, fecal scores were compared within the respective facilities by Kruskal–Wallis test with a subsequent Dunn's post-hoc test (IN-STAT 3, GraphPad Software Inc., San Diego, CA 92130, USA). Regression analysis and Spearman's correlation coefficient (SCC) (SPSS 12.0, Chicago, IL 60606, USA) served to evaluate the relationships among NDF, water content, and the score of the feces. The significance level was set to 0.05.

## RESULTS

Table 1 shows the average quantities of feeds offered and ingested. For diets A, the rice balls supplied were fully ingested, but the pellets, browse, and vegetables were not completely consumed. The langurs given diets B fully consumed the pellets, fruits, and other foods and almost completely consumed the vegetables, but there were leftovers of the browse provided. During periods A<sub>2</sub> and B<sub>2</sub>, the amounts of browse ingested were markedly higher than during periods A<sub>1</sub> and A<sub>3</sub> and periods B<sub>1</sub> and B<sub>3</sub>, respectively. Table 2 shows the nutrient concentrations of the ingested diets.

Contrary to what would be expected, the intake of the various fiber fractions was not markedly raised during periods A<sub>2</sub> and B<sub>2</sub>. Out of the six fiber



**Figure 1.** Feces scoring system for langurs.

measures, only the intakes of crude fiber, NDF, and hemicellulose were slightly, but systematically, increased when vegetables and fruits were removed from the diet. The amount of NSC ingested during periods A<sub>2</sub> and B<sub>2</sub> was not clearly affected. By removal of the produce, dietary water intake dropped markedly. Table 3 shows that the mean feces score was significantly lower when diets A<sub>2</sub> and B<sub>2</sub> were fed, compared to the other diets.

There were significant correlations between the mean fecal score and dietary NDF content (SCC = -0.94;  $P = 0.005$ ;  $n = 6$ ; Fig. 2) and between the mean fecal score and the dietary water content (SCC = 0.87;  $P = 0.019$ ;  $n = 6$ ). A two-variate regression analysis achieved overall statistical significance ( $R^2 = 0.91$ ;  $P = 0.025$ ), indicating that dietary cell wall (NDF) and water content explained a major proportion of the variation ob-



**Table 2.** Dietary dry matter and water intake (g/animal/day) and nutrient composition of the dry-matter fraction of the diets ingested (% of dry matter).

|                           | Diets A        |                |                | Diets B        |                |                |
|---------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                           | A <sub>1</sub> | A <sub>2</sub> | A <sub>3</sub> | B <sub>1</sub> | B <sub>2</sub> | B <sub>3</sub> |
| Dry-matter intake         | 154            | 256            | 268            | 207            | 241            | 238            |
| Water intake <sup>a</sup> | 736            | 468            | 830            | 1,026          | 276            | 1,006          |
| Ash                       | 7.8            | 4.7            | 6.0            | 7.7            | 5.4            | 8.4            |
| Crude protein             | 19.5           | 19.1           | 18.7           | 20.3           | 17.8           | 18.5           |
| Crude fat                 | 5.2            | 3.9            | 5.2            | 5.3            | 7.1            | 7.6            |
| Crude fiber               | 14.3           | 22.3           | 20.5           | 15.9           | 17.4           | 14.7           |
| NDF                       | 34.4           | 41.0           | 37.3           | 31.9           | 39.0           | 35.7           |
| ADF                       | 20.8           | 25.0           | 23.5           | 20.3           | 23.6           | 26.9           |
| ADL                       | 5.2            | 3.1            | 1.1            | 7.7            | 8.7            | 12.6           |
| Hemicellulose             | 13.6           | 16.0           | 13.8           | 11.6           | 15.4           | 8.8            |
| Cellulose                 | 15.6           | 21.9           | 22.4           | 12.6           | 14.9           | 14.3           |
| Nfe                       | 53.2           | 50.0           | 49.6           | 50.7           | 52.3           | 50.8           |
| NSC                       | 33.1           | 31.3           | 32.8           | 34.8           | 30.7           | 29.8           |

<sup>a</sup> Water ingested with food items; does not include drinking water. NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; Nfe, nitrogen-free extract; NSC, nonstructural carbohydrates.

served in the mean fecal score. The regression equation is  $\text{score} = 5.05 - 0.08 \times \text{NDF} + 0.01 \times (\text{water content})$ .

The nonsignificance of the coefficients (NDF:  $P = 0.118$ ; water content:  $P = 0.277$ ) is due to the colinearity between NDF and water content. Thus, this analysis must be regarded as exploratory. Hypothetically, according to this explorative regression, an increase in dietary water by 1% (as fed) would, at constant NDF levels, increase the mean fecal score by 0.01 points. Alternatively, an increase in NDF content by 1% (dry matter basis) would, at constant dietary water content, decrease the mean fecal score by 0.08 points.

## DISCUSSION

This study with Javan langurs in two different zoos demonstrates that the removal of vegetables and fruit from the diet consistently improved feces quality. Clearly, the diet change involved multiple alterations in the intake of nutrients, and, therefore,

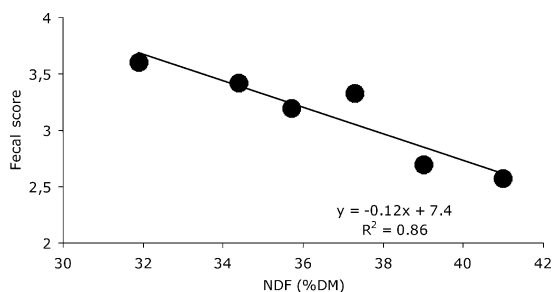
it is difficult to conclude which dietary components affected feces quality. Thus, the present study can only provide additional circumstantial evidence. Furthermore, food intake of individual animals could not be recorded, and differences in food intake between individual animals are therefore not represented in the data. Such differences might be a major cause of the variability in feces quality observed within the individual feeding periods. However, as group composition and the age of the animals did not vary because of the close sequence of the trial periods, the approach of using average intake values is justified.

In an earlier study with François langurs<sup>12</sup> it was shown that an increase in fiber intake led to the production of well-shaped, solid feces. Similarly, in this study, there was a significant negative correlation between the concentration of NDF and fecal score, indicating that higher NDF intakes improved fecal quality. The main reason for this effect can be expected to be a change in the fermentation pat-

**Table 3.** Overview of the feces scoring results for diets A and B.

|                         | Diets A        |                |                | Diets B        |                |                |
|-------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
|                         | A <sub>1</sub> | A <sub>2</sub> | A <sub>3</sub> | B <sub>1</sub> | B <sub>2</sub> | B <sub>3</sub> |
| Number of fecal boli    | 102            | 140            | 100            | 364            | 374            | 376            |
| Mean score <sup>a</sup> | 3.42a          | 2.57b          | 3.33a          | 3.60a          | 2.69b          | 3.19c          |
| SD                      | 0.75           | 0.64           | 0.75           | 0.43           | 0.78           | 0.50           |
| Most common score       | 4              | 2              | 4              | 4              | 2.5            | 3.5            |

<sup>a</sup> Means within a facility with different on-line lowercase letters differ significantly at  $P < 0.001$  (Kruskal-Wallis and Dunn's post-hoc test). SD, standard deviation.



**Figure 2.** Correlation between the mean fecal score and the dietary concentrations of NDF.

tern in the langurs' forestomachs. Because of its high sugar content, produce, and especially fruits, can lead to a very rapid or even "explosive" fermentation.<sup>14</sup> This type of fermentation is when volatile fatty acids—the end products of bacterial fermentation and a major energy source for herbivorous animals—are produced much faster than can be absorbed; as these substances are acidic, this may potentially lead to a drop in forestomach pH, a disruption of the normal symbiotic microflora, and, ultimately, gastric upset, including chronic, intermittent diarrhea, as is observed in cattle with rumen acidosis.<sup>16</sup> In contrast, the slower-fermenting fibers of browse and green vegetables (or wild fruits) will probably induce a slower, more stable fermentation that enhances the natural symbiotic forestomach microflora and will not disrupt the digestive process. Thus, if the correlation found between NDF content and fecal score was considered relevant, one could extrapolate that a dietary NDF content of 46% dry matter (DM) would be necessary to guarantee optimal fecal consistency. This value lies within the range of NDF content provided<sup>13</sup> for both NDF in native colobine foods (ranging from 44 to 67% DM) and in temperate browse (ranging from 44 to 79% DM).

Removal of fruits from the diet may lower the content of soluble sugars. It could be suggested that the decrease in the intake of soluble sugars contributed to the better feces quality observed when diets A<sub>2</sub> and B<sub>2</sub> were fed. The decrease in intake of soluble sugars after feeding diets A<sub>2</sub> and B<sub>2</sub> was not illustrated by chemical analysis of the diets, most likely demonstrating an analytical shortcoming of the methods employed. While it can be expected that the NSC fraction of commercial fruits and vegetables contains mainly sugars, the NSC fraction of browse is more likely to consist of other carbohydrates, such as pectins.<sup>6</sup> Therefore, a change of NDF-soluble carbohydrate sources that would be beneficial for the fiber-digesting symbiotic fore-

stomach microflora due to a higher proportion of browse is likely.

A surprising finding of this study was the concomitant correlation between dietary water content and feces consistency. Although it is often stated by zoo keepers, pet owners, or farmers that the feeding of a fresh (i.e., more watery) forage will result in softer feces, we are not aware of any systematic investigation of this effect. However, it seems that a contribution of dietary water to the fecal score of the langurs of this study cannot be ruled out. In an interspecific eutherian comparison, daily water flux (ml) in mammals equals 0.159 body mass<sup>0.95,18</sup>. Adult individuals of the langur groups studied had recorded body weights between 5 and 6.5 kg. This would result in a theoretical daily water flux of 520–670 ml. In the conventional zoo diets, average daily water intake (740–1,030 ml) surpassed this range considerably. Although one would expect that excess water would be excreted in urine rather than in the feces, it could be speculated that these high water intakes overcharged the animals' capacity for gastrointestinal water absorption.

In conclusion, these trials indicate that the feces consistency of langurs can be improved by an increase of fiber in their diet and that dietary water intake may be a limiting factor, in particular on diets with a high proportion of produce. In order to verify these assumptions, more controlled studies with varying dietary fiber and water levels would be necessary. It should be noted that the recommended feeding practice<sup>5</sup> of a high-fiber primate biscuit, browse, and only limited amounts of green, leafy vegetables will both contain high fiber levels and limit dietary water intake.

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