FIBRE INTAKE AND FAECES QUALITY IN LEAF-EATING PRIMATES

Joeke Nijboer

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CHAPTER 1

INTRODUCTION
AND
SCOPE OF THE THESIS

Introduction

Langurs are also called colobines or leaf-eating monkeys, belonging to the primate subfamily Colobinae. Their natural habitat is Southeast Asia, the area ranging from Southeast China and eastern parts of India as far as Indonesia. Langurs are characterized by their highly specialized digestive system and that they have a large, sacculated stomach containing a variety of microflora which may ferment ingested food (Hill 1964, Bauchop and Martucci, 1968, Napier and Napier 1967, Kuhn 1968, Ohwaki et al. 1974, Kay and Davis 1995, Chivers 1995, Caton 1991, Milton 1998, Hladik 2002). They have a capacious cecum and proximal colon in which fermentation also takes place (Chivers 1995, Kay and Davis 1995, Caton 1991). It would appear that langurs have the capacity to digest and/or ferment plant carbohydrates such as crude fiber, hemicellulose, cellulose, starches and easily digestible carbohydrates.

The natural diet of langurs consist mainly of leaves. The animals prefer especially young leaves. If available, they also eat seeds and fruits. According to Milton (1998), in the wild, sugary fruits are not popular among langurs. Analyses performed on the natural diets of langurs indicate that the average crude protein in the dry matter is 17%, neutral detergent fibre from 44 to 57 %, acid detergent fibre from 31 to 52 % and lignin from 15 to 28%. The high level of lignin implies that young leaves must have represented only a small fraction of the analysed natural diets. Young leaves contain a lower amount of lignin in the dry matter. The water content in the natural diets varies from 5 to 32 % (Nijboer et al. 1997).

The main threat to langurs, which are vulnerable in the wild, is the disappearing of their natural habitat. In nature no problems have been observed in relation to loss of body condition or bad faeces. According to Nadler (1994), faeces consistency in the wild is solid and segmented. Most of the problems associated with langur husbandry in captivity related to the diet. Regularly, poor body condition and loose faeces have been observed (Hölihn 1973, Merrit 1983, Edwards 1990).

Diets for captive wild animals have been developed by trial and error. (Flower 1931, Crandal 1964). The natural diet was assumed to be imitated by offering the captive langurs commercially available substitutes. Due to ignorance of the composition of natural diets, good palatability was the main factor in determining the suitability of the diet (Edwards 1995).

Although the name “fruits” is used in the western hemisphere as well as in tropical areas the composition for the two areas is different (Edwards 1990, Conklin-Brittain and Wrangham 2000, Lawrence et al. 2005). Diets for leaf-eating primates in captivity are widely variable. Often these leaf-eating primates are fed in the same way as their counterparts which are not folivorous and have no pregastric fermentation (Edwards 1995, Hölihn 1973, Watkins et al, 1985). Thus, langurs are fed with high amounts of non-structural carbohydrates and starches and little cellulose. Although many langurs are still fed in this way, nowadays more often the rations are higher in celluloses. This is done by offering special pellets, leaves, vegetables and greens from the western hemisphere, instead of easily digestible carbohydrates that can be found in fruits (e.g. bananas, oranges and apples) and (extruded) primate pellets.

Scope of the thesis

A high incidence of gastrointestinal disorders, such as diarrhea, phytobezoars, bloated conditions, and also weight loss have been observed in captive langurs. This thesis focuses on the effect of the food and nutrient intake (e.g. dry matter, protein, fiber and fat) in captive langurs in relation to the quality of faeces and its implications for langurs kept in captivity.

Chapter 2 presents a review on the physiology of langurs. The chapter describes the function of the compartmentalized stomach. In order to understand the digestion mechanism in langurs it is necessary to describe the breakdown of the plant carbohydrate fraction, the influence of saliva, and that of the digesta passage.

Chapter 3 describes the results of the response on a questionnaire sent to North American and European facilities maintaining langurs. Although quite information could be found in the literature, more information was needed on the current diet status of langurs in captivity. The questions referred to the foodstuffs that were fed according to categories such as fruits, vegetables, animal products, concentrates and vitamins, greens and browse.

As expected, browse forms an important part of the langur diets. However, limited information is available on the browse composition in North America and Europe. Analyses of the temperate browse fed and on the leaf:twig ratio in September, were compared with the samples of July. Attention was given to the amounts of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in browse during the growing season. This study illustrates that currently no standardized diet is fed to langurs in captivity and shows the complexity of the diets fed.

Chapter 4 summarizes the analysed composition of leaves, fruits, flowers and seeds as consumed by proboscis langurs (*Nasalis larvatus*) (Bennet and Sabastian 1988, Yeager 1989), Hose's langurs (*Presbytis hosei*) (Mitchel 1994) and the Ghuihou snubnosed monkey (*Rhinopithecus brelichi*) (Bleisch and Xie 1994) in nature. Furthermore, a comparison was made between the differences in the analysed composition of natural diets and diets fed in zoos. Natural foodstuffs contain more NDF; certain leaves even consist of more than 50% NDF. The degree of lignification was compared between natural and captive diets. The view that diets consumed by langurs in captivity are different from the diets consumed in nature was confirmed.

Chapter 5 describes the effect of additional fiber on the consistency of faeces in a group of four François langurs (*Trachypithecus françoisi*) at Rotterdam Zoo. The experiment followed the so-called A_1-B-A_2 design. In the A periods the normal diet was fed; in the B periods the amount of fiber was increased by feeding a high-fiber pellet. The hypothesis was that stool quality should improve when a high-fiber pellet is fed. During the B period crude fiber intake was almost twice as high as during the control (A_1-A_2) periods. The experimental diet resulted in a lower amount of water intake through the diet and a lower amount of non- structural carbohydrates and it improved faeces consistency.

Chapter 6 describes the effect of introducing a high-fiber pellet on the behaviour of François langurs. It is assumed that a special high-fiber pellet is less palatable than the pellet that is usually given. It was postulated that introducing a high-fiber pellet in the diet could have a significant change in time budget seen during the length of feeding time and the time spent on resting, moving and grooming.

It was also anticipated that the high-fiber pellet could mediate more aggressive behavior. The various expectations was not confirmed. No changes in the time budget were seen. However, the langurs displayed less distinct eating peaks and eating was distributed more evenly over the whole day.

It was considered essential to find out whether the effects of high-fiber diets as described in Chapter 5 would extend to other langur species. Results of digestibility experiments with colobus monkeys (*Colobus guereza*) and spectacled leaf monkeys (*Trachypithecus obscurus*) are given in chapter 7.

Chapter 7. Faeces produced by the colobus monkeys were considered to be well shaped and generally solid. It was concluded that increasing the amount of crude fiber and cellulose in the diet of the spectacled langurs will improve faeces quality.

Chapter 8 illustrates the effect of the intake of produce on the faeces quality in Javan langurs (*Trachypithecus auratus auratus*). Previous studies indicated that a decrease in the intake of non-structural carbohydrates, including soluble sugars, has a positive impact on the faeces quality. This effect could relate to slowing down the fermentation in the gastrointestinal tract of colobines. In this study the effect of removal of produce from the usual diet in two different groups of Java langurs was tested. A firmer faeces consistency interpreted in that correlate with an increase in dietary cell wall, a decrease in dietary water and a decrease in the intake of soluble sugars.

In **chapter 9** the hypothesis was tested that exclusion of feeds rich in soluble carbohydrates (fruits, colored vegetables, cereal products) and an increase of high-fiber pellets and browse would result in a change of the retention time. Two Javan langurs received a diet containing their conventional products (including vegetables and greens) or a diet without these products, only consisting of specialized high-fiber pellets, browse and rice balls.

To estimate the transit time of the liquid and solid phase of the digesta, cobalt-EDTA and chromium oxide were used. A fixed amount of cobalt-EDTA and chromium oxide was put into the rice balls. The mean retention time (MRT) was measured before, during and after feeding, by collecting the faeces of both animals and analysing it for cobalt and chromium.

The diet rich in high-fiber and browse tended to increase the retention times of the fluid and particle part of the digesta. The retention time and the relation between retention time and body mass were compared with observations from another group of foregut fermenters. Improvement of faeces quality was observed during the period in which only browse and pellets were fed which was similar to observations as described in previous chapters. Calculations were made on the dietary water intake during both the conventional and the test period to calculate the water flux in the langurs.

In **Chapter 10**, the hypothesis tested was that feeding of polyunsaturated fatty acids (PUFA) like linoleic acid and α-linolenic acid are hydrogenated into stearic acid and geometrical and positional di- and monoenoic isomers in the stomach of langurs. If hydrogenation does not occur, intact PUFA is absorbed by the intestine which is reflected in a high PUFA content of the erythrocytes and serum of the animals. For this test, langurs were fed with pellets containing soybean oil and linseed oil as a fat source. The results are interpreted in that hydrogenation of linoleic and α-linolenic acid takes places in the stomach of the langurs.

Chapter 11 summarizes data collected from the former chapters and assesses their relevance for the management of foregut fermenters. Results were pooled for 15 feeding periods from 6 feeding trials with colobine monkeys of different species (François langurs, spectacled langurs, black and white colobus and Javan langurs). The same was done for the quality of the faeces. It was found that faeces consistency is negatively influenced by the water content and the amount of protein and positively by the fiber content in the food. For their management in captivity an optimal diet for colobidae, and particularly langurs, should be based on the digestive strategy of these primates, its effect on faeces quality and its palatability of the foodstuffs. Although there are no standards, the diet should be formulated so as to meet the nutrient requirements.

In **chapter 12** the general conclusions of the various studies are listed.

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CHAPTER 2

THE DIGESTIVE PHYSIOLOGY OF COLOBINE PRIMATES

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Summary

In captivity, gastrointestinal disorders such as diarrhea, phytobezoars, bloated conditions and weight loss are frequently observed among colobines. This article describes the processes influencing digestion in colobines.

Colobines are a group of primates from southeast Asia and Africa that feed, in nature, exclusively on leaves, fruits and seeds of trees, which contain high amounts of fibre. Up to 50% of the protein (acid detergent insoluble nitrogen) of their natural food cannot be digested in their stomachs.

The colobine stomach can be divided into the *saccus gastricus*, with or without a *presaccus* – both sites of bacterial fermentation -, and the *tubus gatricus* and *pars pylorica* in which enzymatic digestion is initiated. Bacterial fermentation also occurs in the capacious cecum and proximal colon. The pH and volatile fatty acid content of the different sections of the gastrointestinal tract confirm this pattern of bacterial and enzymatic digestion. To date, no protozoa have been documented in colobine forestomachs although very long ingesta retention times have been measured in several studies in these animals. In general, the existing data are dominated by measurements on captive animals kept on diets that are not representative of the natural diet.

Structural and non-structural carbohydrates are important diet components influencing the microflora in the gastrointestinal tract (GIT). A review is presented on the different carbohydrate fractions.

Natural diets contain higher amounts of acid detergent fibre levels (up to 52%) compared to diets fed in captivity (11-35 %). When digestive efficiency was measured in captive animals fed considerable amounts of browse, with a high lignification of fibre, the resulting values were lower than digestion coefficients measured in colobines fed artificial feeds only.

In colobines dietary fibre levels of neutral detergent fibre and dry matter digestibility are negatively associated, but the slope is moderate which might be consistent with the idea that colobines can ferment dietary fibre efficiently.

Introduction

The objective of this brief and selective review is to set the stage of experiments described in the following chapters. Moreover, various data are taken from the following chapters to illustrate the digestive capacities of colobines. An approach comparing different animal species was not taken because such a description of comparative digestive physiology would be fraught with uncertainly as to whether reported differences are determined by species or diet characteristics. Nevertheless, this review may be interpreted in that colobines can ferment dietary fibre efficiently.

Taxonomy

The Old world monkeys can be divided into two families. Both families, the Colobinae and Cercopithecinae, originate from Africa (Napier and Napier 1967, Medway 1970). In contrast to the monogastric Cercopithecinae, the colobines are characterized by a large, sacculated forestomach with a diverse array of microflora, cheek teeth with increased relief, a reduced first digit on the forelimb, long-distance male vocalisations, flamboyant natal coat and postnatal infant transfer (Bauchop and Martucci 1968, Hill 1964, Kay and Hylander 1978, Kool 1989, Napier and Napier 1967).

Traditionally, the colobines are subdivided according to their present region of distribution into the African “colobus monkeys” (*Colobus spp.*), the Asian “langurs” or leaf monkeys (*Presbytis spp*. and *Trachypithecus spp*.) and the odd-nosed monkeys (*Nasalis spp*, *Pygathrix spp.* and *Rhinopithecus spp*.) (Langer 1988, Kool 1989). Phylogenetic trees of the colobines presented so far are contradictory. A tree based on mitochondrial DNA and the coding region of the lysozyme gene sequence clearly separates the African from the Asian colobines (Messier and Stewart, 1997). In contrast, a phylogeny based on anatomical forestomach characteristics by Caton (1998) found closer relations between the African *Colobus spp*. and the Asian *Semnopithecus*, *Trachypithecus* and *Presbytis* on the one hand – who all have a so-called “tripartite” forestomach that lacks a distinct *presaccus* -, and between the African *Procolobus* and the Asian *Rhinopithecus*, *Pygatrix* and *Nasalis* on the other hand – who all have a distinct *presaccus* at their forestomach, making this organ “quadripartite”.

The discrepancy between the phylogenies based on biochemical and morphological markers suggests that the colobines should be good candidates for the demonstration of convergent evolutionary trends as adaptations to particular challenges to their digestive system.

Primate Species	Plant Part	Water	CP	ADIN	NDF	ADF	Lignin	Field Site
Nasalis larvatus (Proboscis monkey)	Leaves	31.7	11.7	6.1	57.2	46.3	25.8	Bennet and Sebastian 1988
			9.9	3.4	43.7	30.5	14.5	Yeager 1989
	Fruits	78.3	5.2	3.8	66.7	52.3	28.3	Mitchel 1994
	Flowers	37.2	10.4	5.6	50.7	41.6	26.7	Bennet and Sebastian 1988, Yeager 1989
	Seeds	68.4	8.1	3.6				Bennet and Sebastian 1988, Yeager 1989
Presbytis hosei (Hose's langur)	Leaves		16.0	7.4	54.6	42.2	24.4	Mitchel 1994
	Flowers		10.0	7.4	54.6	42.2	24.4	Mitchel 1994
	Seeds		10.9	5.8				Mitchel 1994
Rhinopithecus brelichi (Ghuizhou snub-nosed monkey)	Leaves		14.0	5.3	47.8	37.8	19.5	Bleisch and Xie 1994
	Fruits		5.7	1.5	51.3	40.6	19.9	Bleisch and Xie 1994
Colobus angolensis	Mature Leaves		20.7			24.9	10.5	Nyungwe
Colobus guereza	Mature. Leaves		16.8			19.1	10.7	Kanyawara
	Mature Leaves		18.4		47.3	34.5		Kakamega
	Young Leaves		29.9		35.6	29.4	5.8	Kakamenga
Colobus satanas	Mature.Leaves		7.9			64.7		Douala-Edea
	Young Leaves		5.7			50.7		Douala-Edea
	Seeds		9.1					Douala-Edea
Colobus polykomos	Mature .Leaves		12.4			40.3		Tiwai
	Young Leaves		22.3			26.9		Tiwai
	Unripe seeds/fruits		22.4			24.9		Tiwai
Procolobus badius tephrosceles	Mature.Leaves		18.4			35.1	19.8	Kanyawara
	Mature Leaves		16.4			33.0		Dura River
	Mature Leaves		18.3			35.0		Mainaro
	Mature Leaves		17.0			36.4		Sebatoli
	Young Leaves		37.0			22.8	12.3	Kanyawara
Procolobus badius rufomitratus	Mature Leaves		13.3			29.6		Mcheleo
	Young Leaves		20.1			23.8		Mcheleo
	Mature Fruits		6.9			50.7		Mcheleo
	Immature Fruits		7.3			57.4		Mcheleo
	Flowers		16.3			24.2		Mcheleo
Prcolobus verus	Mature Leaves		13.5			51.9		Tiwai
	Young Leaves		22.1			22.0		Tiwai
	Seeds	20.6				50.7		Tiwai

Abbreviations: CP = crude protein; ADIN = acid detergent insoluble nitrogen; NDF = neutral detergent fiber; ADF = acid detergent fiber

Table 1: Summary of the chemical composition of native food plants eaten by southeast Asian colobines and Colobus monkeys according to Nijboer et al. (1997) and Lawrence et al. (2005). All nutrient contents are expressed on a dry matter basis except water in percentage.

Natural diet

The natural diet of colobines has been reviewed by Nijboer et al. (1997) and Lawrence et al. (2005) and is summarized in Table 1. It consists exclusively of leaves, fruits and seeds of trees and is therefore of a high fibre content. Insects that are ingested together with plant material (Srivastava 1991) should not be overestimated in their nutritional contribution to the overall diet.

The reported protein content of the food items appears moderate to high, but it should be remembered that a proportion of the analysed nitrogen stems from lignified cell walls (acid detergent insoluble nitrogen = ADIN) and is hence unavailable for digestion in the stomach (Van Soest 1994). A better approach to the determination of the available protein therefore is a combination of the total nitrogen and the acid detergent fibre bound nitrogen; the difference between these two measures will more likely reflect the really available protein for fermentation in the stomach. Ideally, digestibility trials should be carried out to asses the amount of digestible in foodstuffs. Nijboer et al. (1997) and Lawrence et al. (2005) summarize data showing that the amount of ADIN measured in this way can amount up to 50 % of the total protein value. Another important component of the natural diet of colobines are the secondary plant compounds present mainly in leaves, such as tannins. A large number of publications refers to this phenomenon (Oates et al. 1980, Mckey et al. 1981, Moreno-Black and Bent 1982, Davies et al. 1988, Kool 1992, Mowry et al. 1996, Burgess and Chapman 2005). The relevance of these substances is that they will reduce overall digestibility or act as toxins; therefore, they are feeding deterrents that will influence diet choice. On the other hand, they could have some potentially beneficial effects, such as a suppression of pathological gastrointestinal bacteria and parasites as reviewed by Clauss (2003). The fact that phenolic secondary compounds are not strictly avoided by colobines (Kool 1989, 1992) indicates that these animals are equipped with mechanisms to deal with such substances. The relatively large salivary glands of colobines (Kay et al. 1976) could be an indication that they can produce tannin-binding salivary proteins (Clauss 2003). Such a mechanism operates in ruminants. Another possible defence mechanism is the ingestion of clay that could bind to, and thus reduce the effect of tannins (Muller 1996, Oates 1978).

When colobines are transferred from the wild to captivity, the diets they are offered are mostly of lower fibre content. The captive diet consists of low-fibre foods rich in easily fermentable carbohydrates and often contains diet items rich in available protein (such as dairy products, eggs, or even meat). The diets fed to captive colobines usually are low in secondary plant compounds (Hölihn 1973, Nijboer and Dierenfeld1996).

The gastrointestinal tract (GIT)

Fig. 1 shows a photograph of the complete GIT of a François langur (*Trachypithecus françoisi*) taken from an individual that died at Rotterdam Zoo. Fig. 2 gives a graphic visualisation of the total GIT of a Colobus monkey (*Colobus abyssinicus*) published by Stevens and Hume (1995). Fig. 3 shows an overview of the GIT of a dusky langur (*Trachypithecus obscurus*) according to Caton (1999).

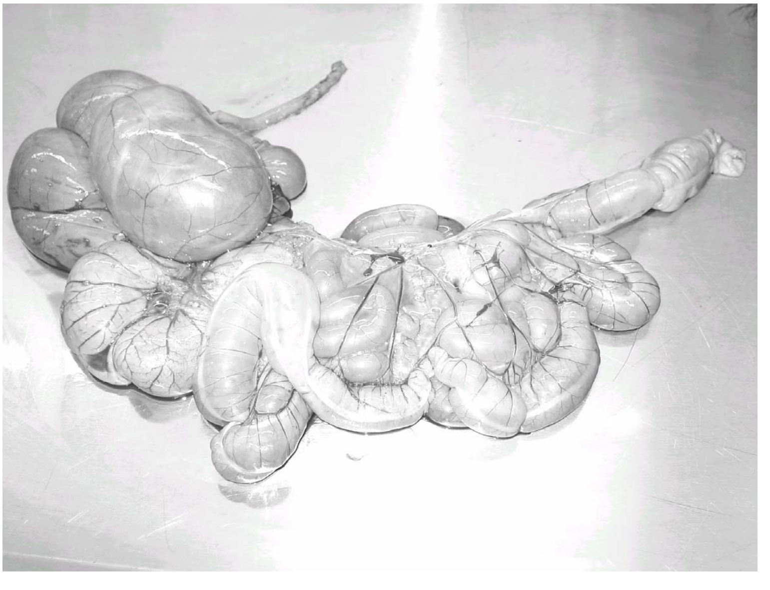

Figure 1: Picture of the digestive tract of a François langur (*Trachypithecus françoisi*)

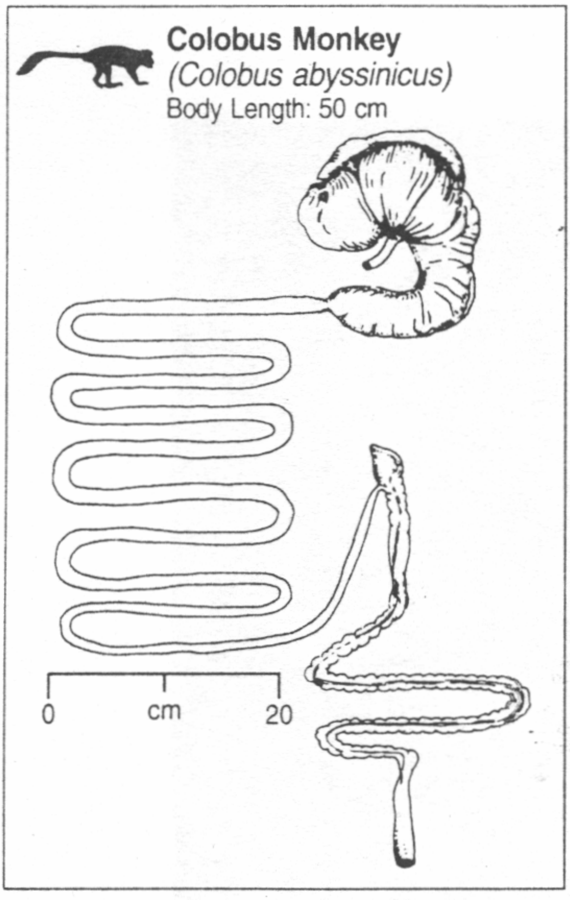

Figure 2: Graphic visualization of the digestive tract of a colobus monkey (Stevens 1995).

Like other forestomach fermenters – including the well-investigated domestic ruminants – colobines have a multichambered forestomach where food is fermented. The anatomy of this compartment has been described in most detail by Langer (1988) who also reviews the older anatomical literature. The first section is a voluminous compartment (*saccus gastricus*, with or without a *presaccus*), lined with a cardiac glandular mucosa, where food is fermented. This compartment is linked by a *tubus gastricus*, the last part of which is lined with gastric gland mucosa, to the *pars pylorica* with pyloric mucosa. This final part then empties into the first part of the small intestines, the duodenum (Bauchop and Martucci 1968, Langer 1988, Chivers 1995, Caton 1990). Apart from this voluminous stomach complex, colobines have a capacious caecum and proximal colon (Kay et al. 1976, Kay and Davies 1994, Chivers 1995) in which additional bacterial fermentation takes place (Caton 1990).

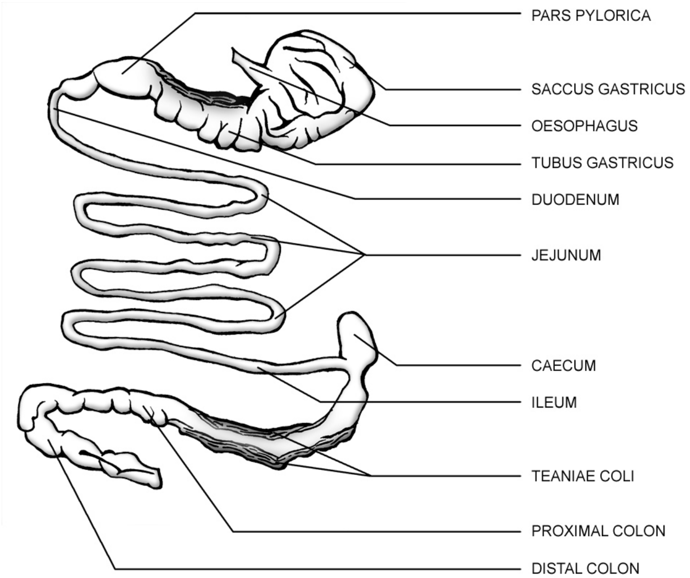

Figure 3: Schematic overview of a the digestive tract of a dusky langur (Caton 1999).

It has been noted that compared to other foregut fermenters, the colobine forestomach is of a relatively small capacity (Langer 1988). Forestomach contents comprise mostly 6-8 % of the body weight (Kay and Davies 1994). Chivers (1995) claims that essentially folivorous colobines have more voluminous forestomachs than do the essentially frugivorous colobines, which in turn have a longer small intestine.

The pH in the forestomach compartment varies between 5.0 and 7.0 according to different publications, but it appears that the lower values were found in animals on concentrated diets (Kay and Davies 1994). In a comparative survey on the forestomach pH in apparently healthy animals and those with a clinical history of gastrointestinal upset, Sutherland-Smith et al. (1998) found a pH range of

6.5-8.0 in the healthy and of 4.5-8.5 in the abnormal animals. Possibly values below 6.0 point at an abnormal state. The pH starts to drop to values of 2.4 in the *tubus gastricus*, indicating true acidic stomach digestion in this region (Kay et al. 1976).

Volatile fatty acid production has been measured in the *saccus gastricus* (Drawert et al. 1962, Bauchop and Martucci 1968, Kuhn 1968, Ohwaki et al. 1974, Kay et al. 1976). In those publications where the *tubus gastricus* was also sampled, it became evident that volatile fatty acid production drops markedly in this organ, again indicating the onset of acidic, peptic digestion at this site. Kay et al. (1976) analysed contents of the caeco-colon and found a volatile fatty acid concentration that was higher than found in the forestomach, underlining the concept that fermentative digestion takes place at both sites in these animals (Caton 1990). However, it should be noted that the animals killed for the pH measurements were mostly captive ones, sometimes should be noted not thriving well before the measurements were made. In two animals taken directly from the wild, Ohwaki et al. (1974) measured a C2:C3-ratio of 3.2 indicating predominantly fibre fermentation. In contrast, Bauchop and Martucci (1968) used animals fed with a diet consiting of unknown proportions of lucerne, yam, beens and cereals. They found C2:C3-ratios of 1.4-2.3, which are indicative of predominant starch fermentation. The author measured pH values below 5.5 in 3 out of 8 animals. The low pH values could point at rapid fermentation introducing a risk of acidosis. No protozoa were found in the forestomach of the animals investigated by Bauchop and Martucci (1968). This is an interesting finding, especially considering the long ingesta retention made in colobines (Nijboer et al. 2006c) which would allow enough time for protozoal generations to establish themselves. However, a confirmation of this finding in free-ranging animals or animals kept on a natural diet is lacking.

Digesta passage

Several authors have noted that colobine primates have surprisingly long fluid and particle retention times in relation to their body size (Nijboer et al. 2006c). A data collection, including measurements by the first author as part of this thesis, are summarized in Figure 4. Note that in most studies, the particle mean retention time (MRT) was well above 40 hours. Such long retention times should allow smallbodied primates enough time to ferment the fibrous components of their herbivorous diet efficiently.

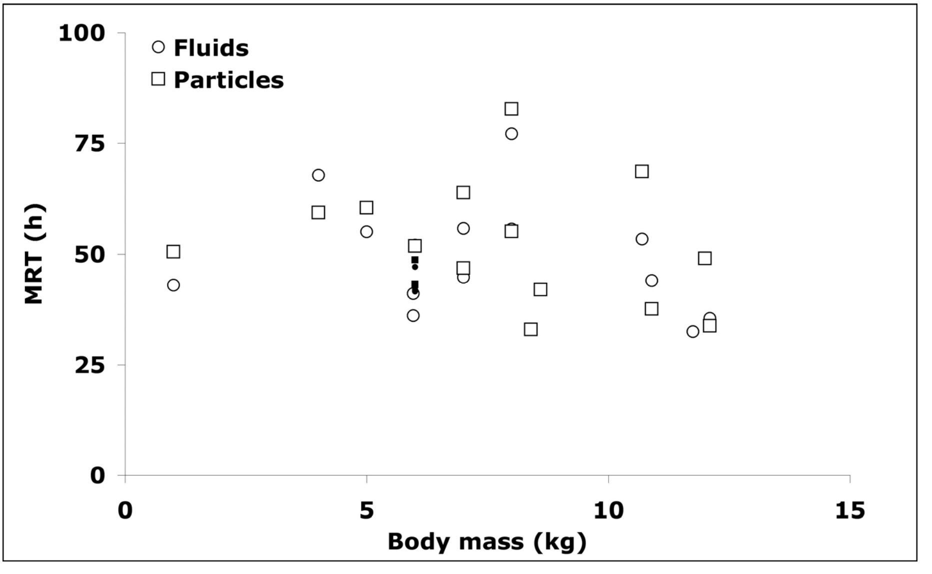

Figure 4: Comparing retention time with body size in langurs. Data from Dierenfeld et al. (1992), Edwards and Ullrey (1999), Caton (1999), Kirkpatrick (2001), Sakaguchi. (1991). Data from Nijboer et al. (2006c) are indicated by black symbols.

It has been speculated recently that mechanisms of flotation and sedimentation could operate in primate foregut fermenters including the effect of separating larger and smaller particles (Claus 2004). The similarity in fluid and particle passage in colobines (Figure 4) suggests a similar lack of phase differentiation in the colobine forestomach. A possible explanation for this lack of selective particle retention could lie in the conentration of the foregut contents, as a viscous environment will prevent sedimentation and flotation effects from becoming effective (Clauss et al. 2006). Colobines seem to have particularly large salivary glands (Kay et al. 1976), and Sutherland-Smith et al. (1998) describe the normal foregut contents as “viscous”. Analyses of the forestomach ingesta of two free-ranging animals by Ohwaki et al. (1974) indicated a dry matter content of 18.7 and 31.7%, respectively. Evidently, in such a relatively dry medium, a separation of different particle sizes would appear difficult. To date, no studies on colobine saliva, or on ingesta passage with different markers for different particle sizes, have been published.

Structural and non-structural carbohydrates

Langurs have a specialized stomach system in which nutrients and specific fibres can be digested. Fibre is an important part of plants; cellulose forms the main structural component in all plants. For future differentiated understanding of the digestion mechanisms in herbivores, it has to be clear in which components fibre can be fractionated.

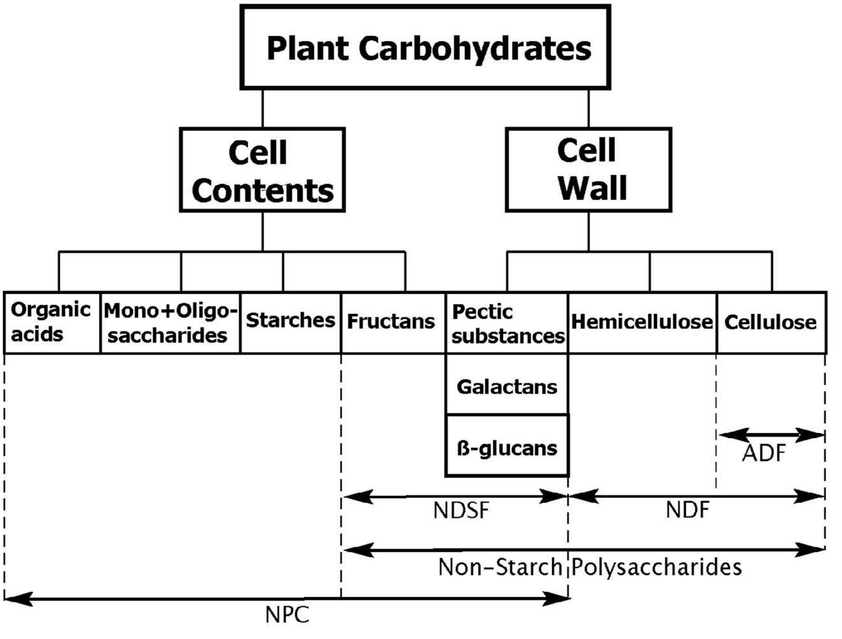

Figure 5: Plant carbohydrate fractions (Hall 2002).

Figure 5 describes the plant carbohydrate fraction. The primary cell walls contain 3.000 to 5.000 glucose residues in a single chain of cellulose. Secondary cell walls contain 20.000 glucose residues (Smith 1999). The glucose residues are joined creating straight, unbranched chains of polysaccharids; 36 of them create a microfibril (Carpita 1987) which is interwoven in the plant cell wall to establish the structural stability. Hemicellulose is a polysaccharide consisting of numerous monoscharrides and is capable of crosslinking cellulose microfibrills in the primary cell wall through H-bonding (Schmidt 2002). The main hemicellulose components are xyloglucan, xylan and glucuronarabinoxylan. Pectin is also a part of the structural fibre network.

Another portion of the fibre is lignin which cannot be hydrolised by enzymatic processes, except by certain species of fungi (Schmidt 2002). Lignin is a hydrophobic polymer, which is recognised as a phenolic compound. These phenolic compounds can act as a cross-links between polysaccharids (Fry 1988). Figure 5 does not include lignin, stressing the fact that in chemical terms lignin is not a carbohydrate.

Neutral detergent fiber consists of hemicellulose, cellulose and lignin, of which hemicellulose and cellulose can be degraded by the symbiotic gastrointestinal microflora. Hemicellulose is calculated as NDF - ADF. Acid detergent fiber is composed of cellulose and lignin. Cellulose is calculated as ADF – Lignin. The cell contents consists of proteins, lipids, and of organic acids, mono- and oligosaccharides, starches, fructans and partly of the pectic substances galactans and β-glucans. Non-structural carbohydrates (NSC) are calculated as 100 - crude protein – neutral detergent fiber – ether extract – ash (Hall 2002). This fraction is an important source of energy in rations of high-output dairy cattle, however, this fraction, especially starch, has been associated with rumen acidosis (Sutton et al. 1987, Nocek 1997).

Organic acids are not true carbohydrates. They include fermentation acids found in silage and plant organic acids found in fresh forage and hay, and can be digested by mammalian enzymes. Sugars contain monosaccharides, like glucose, fructose and sucrose, and oligosaccharides. Sugars tend to ferment rapidly and may ferment into lactic acid. Starch is composed of alpha-linked chains of glucose that in plants are stored in crystalline granules. Fermentation rates depend on the processing, the storage method, and the plant source.

The fructans, pectic substances, galactans and β-glucans are called neutral detergent soluble fibres. These fibres cannot be digested by mammalian enzymes but are fermented by microbes. Soluble fibres tend to ferment very rapidly (Hall 2002). According to Schmidt (2002), vegetables and leafy greens provide high amounts of NDF (19 %DM) whereas fruits do not (13 %DM). Starch levels are higher for fruits than for vegetables or leafy greens. Fruits are highest in free sugars. It is anticipated that high intakes of rapidly fermentable carbohydrates may lead to a drop of pH in the forestomach which in turn will slow down fermentation. Thus, it could be suggested that the diet of colobines should be well balanced with regard to fibres and easily digestible carbohydrates. The optimum diet composition for captive colobines so as to improve and maintain their health status is not yet known.

Digestion

A limited number of studies on the digestive efficiency of colobines has been published (Oftedal et al. 1982, Watkins et al. 1985, Sakaguchi et al. 1991, Dierenfeld and Koontz. 1992, Edwards and Ullrey 1999). The most remarkable characteristics of these studies – with the exception of that of Edwards and Ullrey (1999) – is that the fibre levels of the diets used, measured as acid detergent fibre (ADF), were relatively low (9-15 %DM). The high fibre-pellet used by Edwards and Ullrey (1999) reached ADF levels of 30 %DM. Compared to ADF levels of up to 52 %DM in the natural diet, these experimental diets must therefore be considered low in fibre. In this respect, the digestion studies performed by the first author in the course of this thesis not only represent an expansion of the existing database from 11 feeding trials reported in five publications by another 9 feeding trials (Nijboer et al. 2001, Nijboer et al. 2006a, 2006b, 2006c, 2006d), but also a higher range of ADF levels (12-35 %DM).

Another important difference between the trials reported in the literature and those performed for this thesis is that in the literature reports all referred to diets based on pelleted feeds (and produce), but not to natural browse. In contrast, the studies of Nijboer et al. (2001, 2006a, 2006b, 2006c, 2006d) all used rations containing a certain proportion of browse which will, due to a higher lignification of the fibre fraction, and the presence of secondary plant compounds, lead to lower overall and fibre digestion coefficients. As can be seen from Table 1, the digestion coefficients measured during the project described in this thesis extend the lower range of measurements of the published literature. Nevertheless, the high efficiency of fibre digestion in colobines remains the most remarkable finding of this data compilation. And although the high lignin digestion coefficients measured by Dierenfeld and Koontz (1992) appear as outliers, the data in Table 2 indicate that ingested lignin disappears and is not recovered in faeces pointing at apparent digestion. That lignin would be apparently digested is at variance with current knowledge of digestive processes. Methodological error might be an explanation. In early event, the fermentation activity in the colobine GIT changes the cell wall components enough to result in a numerical disappearance of lignin. Evidently, lignin cannot be used as an internal digestion marker in colobines.

Table 2: Digestion coefficients for colobines as taken from different publications.

Source	Species	DM	CP	NDF	ADF	ADL
Oftedal et al. (1982)	*Colobus guereza*	83	83	68	68	31
Watkins et al. (1985)	*Colobus guereza*	87	78	81	69	21
Sakaguchi et al. (1991)	*Semnipithecus cristatus*	76	83	69	62	
Dierenfeld and Koontz (1992)	*Nasalis larvatus*	88		86	85	82
Dierenfeld and Koontz (1992)	*Nasalis larvatus*	89		86	86	82
Edwards and Ullrey (1999)	*Colobus guereza*	80		77	80	
Edwards and Ullrey (1999)	*Colobus guereza*	79		74	56	
Edwards and Ullrey (1999)	*Pygathrix nemaeus*	76		67	67	
Edwards and Ullrey (1999)	*Pygathrix nemaeus*	74		70	68	
Edwards and Ullrey (1999)	*Trachypithecus françoisi*	82		79	82	
Edwards and Ullrey (1999)	*Trachypithecus françoisi*	77		76	77	
Nijboer et al. (2001)	*Trachypithecus françoisi*	59	50	47	38	25
Nijboer et al. (2001)	*Trachypithecus françoisi*	65	68	62	56	36
Nijboer et al. (2001)	*Trachypithecus françoisi*	74	70	68	73	44
Nijboer et al (2006d)	*Trachypithecus obscurus*	78	81	69	63	33
Nijboer et al (2006d)	*Trachypithecus obscurus*	88	70	74	54	49
Nijboer et al (2006d)	*Colobus guereza*	67	56	60	56	46
Nijboer et al. (2006c)	*Trachypithecus auratus aratus*	67	65	54	54	35
Nijboer et al. (2006c)	*Trachypithecus auratus aratus*	61	43	44	48	11
Nijboer et al. (2006c)	*Trachypithecus auratus aratus*	69	62	55	55	39

Abbrevations: CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin.

In various animal species there tends to be a lowering effect of increasing dietary crude fibre concentrations (CF %DM) on apparent organic matter digestibility (aD OM). From the data generated by Nijboer et al. (2001, 2006a, 2006b, 2006c, 2006d), the following equation can be derived for colobines: aD OM = 88 – 0.89 CF (n=9, R^2=0.49), also indicating that an increase in fibre content effects a reduction of aD OM. Clearly, the relationship hold for a limited range of crude fibre levels and also for the diet formulations used. Similarly, a graph combining all available data on dietary fibre levels, measured as neutral detergent fibre (NDF), and dry matter digestibility (aD DM), indicates a moderate slope of the relationship (Figure 6). The optimum diet composition for captive colobines so as to improve and maintain their health is not yet known. Colobines are fibre fermenters, and their diets in captivity should reflect this.

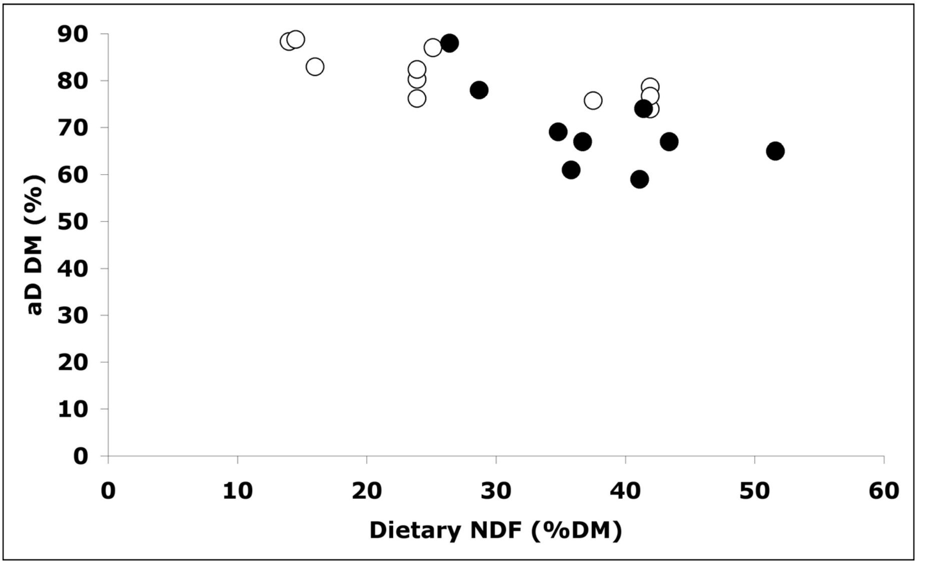

Figure 6. The relationship between dietary fibre content (measured as neutral detergent fibre, NDF) and apparent dry matter digestibility (aD DM) as based on iterature values (Oftedal et al. 1982, Watkins et al. 1985, Sakaguchi et al. 1991, Dierenfeld and Koontz 1992, Edwards and Ullrey 1999 = open symbols) and the studies performed for this thesis (Nijboer et al 2001, 2006c 2006d = black symbols).

Conclusions and outlook

Captive colobine monkeys are prone to a range of diseases that are all related to the gastrointestinal tract, but that have rarely been diagnosed in detail (Janssen 1994). Consistency of the faeces in captive colobines regularly differ from the faeces consistency of langurs in the wild. However, it is an important factor in the measurement of the health status of colobines. Differences in faeces consistency, compared to the natural faeces consistency in colobines, can result in diarrhoea, bloated conditions and weight loss (Nijboer et al. 2006a). It is suggested that it is caused by disturbance of the digestion processes in the colobines gastrointestinal tract.

It has been speculated repeatedly that the feeding of high-sugar or high-starch items, such as fruits and cereal products, could lead to a condition in colobines similar to rumen acidosis in cattle, and the low pH values measured in some captive animals (Bauchop and Martucci 1968, Sutherland-Smith et al. 1998) support this view. Bloated conditions may be associated with rapid fermentation (Hollihn 1973), as well as weight loss and gastrointestinal discomfort. However, in order to reduce rapid fermentation the feeding of any type of browse might be not suitable for colobines. There is suggestive evidence for the occurrence of phytobezoars in the colobine stomach, due to the use of browse items that these animals are not adapted to (Ensley et al. 1982, Janssen 1994, Calle et al. 1995) Whether foregut fermentation provides these monkeys with a digestive advantage that could not be obtained otherwise, or whether their digestive strategy should just be considered a historically possible solution to folivory, remains to be investigated.

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CHAPTER 3

Comparison of diets fed to Southeast Asian colobines in North American and European zoos, with emphasis on temperate browse composition.

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Abstract

In May and June 1994 a survey of diets fed to south-east Asian colobines in captivity in European (n = 12) and North American (n = 9) zoos was conducted. Most diets were very complex, comprising an average of 25 ingredients; 149 different foods were listed in responses. Comparison of diets fed showed that European zoos feed a greater variety of fruits and vegetables, and fewer browse plants, than North American zoos. No standardized diet recommendations, based on ingredient or nutrient composition, are currently available for colobines in captivity.

Foods eaten by these primates in nature appear to contain higher amounts of fibre and lower protein and soluble carbohydrates than current zoo diets. Temperate browse plants (n = 11)sp.; leaves plus twigs) samples in New York in summer and autumn contained higher fibre and lower protein levels than diets fed in this survey, and may approximate the nutrient content of food items selected by free-ranging Colobines. Fast-growing roses grown in greenhouses, fed primarily in Europe, contained substantially less fibre and higher protein concentrations than other browse offered, and may not be an appropriate substitute for native foods.

Key words: browse composition, die survey, colobine nutrition

Introduction

As of June 30, 1994, 40 zoos recorded 14 species of subspecies of southeast Asian colobines in worldwide collections, comprising a total of 321 animals. Nineteen young were born in captivity over the past 12 months, with 15 belonging to only three species or subspecies [ISIS, 1994], Populations of these primates both in zoos and in nature are threatened, and require focused attention.

Colobines have a highly specialized digestive system [Kuhn, 1968; Ohwaki et al., 1974; Kay and Davies, 1994]. Digestive disturbances have been identified as a major health problem in captivity [see Janssen, 1994; Calle et al., 1995], which may be related to an inappropriate diet. Based on survey data from 12 European and nine North American zoos, representing 48% (n = 160) of the extant zoo population at the time [ISIS, 1993], diets offered in captivity contained substantially more soluble carbohydrate and available protein, and less fibre than plants, consumed by free ranging colobines [see Kay and Davies, 1994; Waterman and Kool, 1994] [Nijboer et al., unpublished observations].

Limited information is available on the nutrient composition of temperate browse plants fed to these primates in zoos for comparison with natural forages. This paper focuses on specific differences between diet ingredients in European and North American zoos, and further documents seasonal differences in chemical constituents of selected browses commonly used in North American facilities. Such information, when combines with data from natural diets and feeding obeservations, may be integral in the development of optimal diets for managed populations of these species.

Materials and Methods

Diet Comparison

A survey was sent to 15 European and 17 North American zoos holding south-east Asian colobines in their collections. Respondents provided dietary information (ingredients and amounts offered), which was entered into a commercial software program (Animal Nutritionist, N^2Computing, Silverton, OR) for initial evaluation.

Chemical Analysis of Browse

Samples of 11 temperate browses (sampled from 3 – 5 individual trees) commonly fed in North America facilities and rose browse from The Netherlands were analyzed in the Nutrition Laboratory, Wildlife Conservation Society, Bronx, NY. Fresh weights of leaves and twigs were taken, and samples dried at ≤ 60ºC to obtain moisture content; leaf:twig ratios were determined on an as-sampled (wet) weight basis. Dried plants samples were ground in a Wiley laboratory mill through a 2 mm screen, and analyzed according tot AOAC methods for animal feeds [Jones, 1984].

Crude protein (CP) values were determined as total nitrogen x 6.25 using a macro-Kjeldahl method with a copper catalyst. Acid detergent-nitrogen x 6.25 (AC-CP) was evaluated as a measure of unavailable protein [Goering and Van Soest, 1970]. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and sulphuric acid lignin (Lig) values were quantified through sequential analysis using the methods of Goering and Van Soest [1970], with no pre-treatment of enzymes. Total ash content was obtained by burning samples (0.5 g) at 550ºC overnight in a muffle furnace.

Paired comparisons of nutrient concentrations in leaf vs. twig fractions, or species x season, were performed using the SYSTAT computer software package [Wilkinson, 1987].

Results

Captive Diet Survey

Twelve of 15 European zoos (80%) and nine of 17 North American zoos (53%) replied to the survey; data represent diets offered to 48% of the captive southeast Asian colobine population at the tie (n = 160/ animals). Table 1 shows the different species of southeast Asian colobines distributed in European and North American zoos that were involved in this project.

Table 1. Distribution of southeast Asian colobines comprising diet survey results*

Species	Europe Total # Zoos	Europe Total # Animals	N. America Total # Zoos	N. America Total # animals
Thrachypithecus auratus sp.	4	31	--	--
T. obscura sp.	3	27	3	14
T. cristatus sp.	3	34	2	21
T. françoisi	1	3	3	14
Nasalis larvatus	1	2	1	5
Pygathrix nemaeus	1	11	--	--

*Survey results represent 48% of total captive population (in these continents).

Zoo Diets

One hundred an forty-nine different diet ingredients were fed among the surveyed zoos, including 50 browses, 35 commercial products, concentrates and/or vitamin/mineral supplements, 22 vegetables, 18 fruits, 18 greens, and six animal-based products (Nijboer et al., unpublished’. A summary of foods most frequently fed (n = 28) in the 21 responding zoos can be found in Table 2. Because many zoos did not know the exact quantity of browse fed, browse as a percentage of the diet in relation to the total amount of other food categories could not be calculated for most facilities. Response frequency for various food items was selected as a relative modal statistic for comparison of differences in diets offered between European and North American zoos.

Foodstuffs listed by > 1 but ≤ 4 zoos include: kiwi, melon, mulberry, and orange (fruits); aubergine, avocado, beets, cauliflower, corn (sweet), leeks, peas, potato (sweet), and zucchini (vegetables); cottage cheese, curd and honey (animal products); peanuts, rolled oats, rice, sunflower seeds, and wheat bran (concentrates, grains, nuts); endive and parsley (greens); apple tree leaves, bamboo, beech, black currant, birch, cotoneaster, coprosma, grape vine, hawthorn, hazel, honeysuckle, lime, lilac, oak, plane tree, and privet (browses); and fennel tea (miscellaneous).

Table 2. Most frequently used foodstuffs in colobine diets at European and North American zoos (used in > 4 facilities)

	Europe		N. America	
	# zoos responding	Response frequency[a]	# zoos responding	Response frequency[a]
Fruits		0.6		0.3
Apple	7	0.6	5	0.5
Banana	10	0.8	4	0.4
Grape	7	0.6	1	0.1
Pear	4	0.3	1	0.1
Vegetables		0.5		0.2
Bean, green	1	0.1	4	0.4
Carrot	8	0.7	5	0.5
Celery	9	0.8	2	0.2
Cucumber	7	0.6	0	0.0
Onion	5	0.4	2	0.2
Pepper, green	6	0.5	1	0.1
Potato	6	0.5	1	0.1
Tomato	6	0.5	1	0.1
Yam	0	0.0	6	0.6
Animal products		0.5		0.1
Egg	6	0.5	1	0.1
Concentrates/ Vitamins:		0.5		0.5
Bread	6	0.5	0	0.6
Pirmate pellet	8	0.7	6	0.6
Vitamins	4	0.3	2	0.2
Greens		0.4		0.4
Broccoli	3	0.3	3	0.3
Cabbage	4	0.3	1	0.1
Fennel	8	0.7	0	0.0
Kale	1	0.1	6	0.6
Kettuce	7	0.6	2	0.2
Spinach	1	0.1	6	0.6
Browse		0.4		0.2
Elm spp.	3	0.3	2	0.2
Maple spp.	4	0.3	2	0.2
Poplar spp.	4	0.3	1	0.1
Rose	6	0.5	0	0.0
Willow spp.	7	0.6	3	0.3

[a] Response frequency = (total # responses/ total # zoos surveyed).

Table 3. Chemical composition of common browses fed in the Wildlife Conservation Park, Bronx, NY (July 1994 samples)

	L:T[a]	Water	Crude P	AD-CP[b]	NDF	ADF	Lig	Ash
Family/species	%		------------------------ % of dry matter --------------------------					
Aceraceae *Acer negundo*	L	72.9	15.6	1.8	30.9	21.6	6.7	8.4
Box Elder	T	59.0	8.1	1.5	67.9	54.0	19.2	4.4
	57:43	66.9	12.4	1.7	46.8	35.5	12.1	6.7
A. saccharinum	L	50.9	15.4	1.0	19.8	14.7	6.0	7.1
Silver Maple	T	49.0	4.3	1.5	63.8	46.8	12.5	4.3
	57:43	50.1	10.7	1.2	38.7	28.5	8.8	5.9
A. saccharum	L	57.8	13.3	2.3	29.0	20.8	8.1	8.2
Sugar Maple	T	45.5	4.3	2.9	62.7	51.1	20.8	7.0
	47:53	51.3	8.5	2.7	45.9	36.8	14.9	7.6
Fagaceae Fagus sp	L	64.5	15.8	5.7	61.1	32.4	10.9	6.5
Beech	T	54.1	3.3	1.6	84.0	79.2	25.1	2.8
	39:61	58..1	8.2	3.4	75.1	61.0	19.6	4.3
Hamamelidaceae *Liguidambar sp.*	L	64.6	11.6	3.4	40.0	35.4	19.9	2.7
Sweet Gum	T	54.6	3.6	2.6	64.1	51.1	19.9	5.2
	54:46	60.0	7.9	3.0	51.1	42.7	19.9	3.8
Moraceae *Morus sp*	L	65.9	18.9	1.1	21.3	14.4	4.5	15.1
Black Mulberry	T	55.8	5.9	2.0	60.3	48.7	18.0	7.1
	42:58	60.0	11.3	1.6	44.0	34.3	12.3	10.4
Salicaceae *Populus alba*	L	68.0	17.4	2.7	41.7	27.0	11.0	13.0
White Poplar	T	64.2	5.3	1.8	61.2	59.2	26.0	7.6
	63:37	66.6	12.9	2.4	48.9	38.9	16.5	11.0
Salix babylonica	L	63.5	18.0	2.1	29.6	24.8	14.6	12.8
Weeping Willow	T	50.0	2.8	1.9	69.7	60.2	20.4	3.8
	64:36	58.6	12.5	2.0	44.1	37.6	16.7	9.6
Salix nigra	L	62.6	18.3	2.8	38.7	27.0	13.4	8.9
Black Willow	T	53.4	6.6	2.2	61.4	50.5	20.1	5.6
	62:38	59.1	13.9	2.6	47.3	35.9	16.0	7.7
Ulmaceae *Ceitis occidentalis*	L	62.6	16.1	1.3	36.1	20.4	7.5	8.7
Hackberry	T	40.4	5.0	2.1	74.5	53.0	16.9	7.0
	54:46	52.4	11.0	1.7	53.8	35.4	11.8	8.0
Vitaceae *Vitis sp.*	L	79.1	16.7	0.6	19.9	14.7	5.5	11.2
Grapevine	T	84.8	3.0	2.3	70.2	55.2	16.7	5.6
	50:50	80.4	9.9	1.5	45.1	35.0	11.1	8.4

[a] [a]L = Leaf; T = Twig; Leaf:Twig proportions (% wet weight) of whole plant sample as offered.
[b] [b]AD-CP = acid detergent crude protein (bound protein); NDF = neutral detergent fiber; ADF = acid detergent fiber; Lig = sulphuric acid lignin.

Table 4. Chemical composition of common browses fed in the Wildlife Conservation Park, Bronx, NY. (September 1994 samples)

	L:T[a]	Water	Crude P	AD-CP[b]	NDF	ADF	Lig	Ash
Family/species			------------------------ % of dry matter					-------------------------
Aceraceae *Acer negundo*	L	74.8	19.1	1.0	28.5	23.4	5.9	1.8
Box Elder T	T	50.4	5.7	1.7	83.0	68.3	22.2	3.4
	29:71	57.4	9.5	1.5	67.4	55.3	17.5	8.9
A. saccharinum	L	70.1	18.4	3.3	50.1	35.6	8.9	9.9
Silver Maple	T	67.8	5.4	1.7	79.2	65.1	22.4	4.6
	44:56	73.9	11.1	2.4	66.4	52.1	16.5	6.9
A. saccharum	L	46.2	14.4	1.6	38.6	24.1	3.5	4.3
Sugar Maple	T	41.5	4.9	1.9	75.3	60.8	19.5	3.0
	48:52	43.8	9.4	1.8	57.8	43.3	11.9	3.6
Fagaceae *Fagus sp*	L	55.0	16.0	3.2	72.8	39.8	14.4	7.0
Beech	T	40.4	3.8	1.9	82.7	68.0	23.9	1.1
	39:61	37.8	8.6	2.4	78.9	57.0	20.2	3.6
Hamamelidaceae *Liguidambar sp.*	L	82.3	10.6	3.1	39.6	32.5	15.5	6.0
Sweet Gum	T	47.9	2.9	1.6	68.3	57.9	21.6	7.5
	25:75	56.6	4.8	2.0	61.1	51.5	19.9	7.2
Moraceae *Morus sp*	L	55.0	19.0	1.1	32.6	21.4	4.1	20.7
Black Mulberry	T	46.8	4.7	1.4	75.9	61.0	17.7	5.2
	43:57	50.4	10.9	1.3	57.2	43.9	11.8	11.9
Salicaceae *Populus alba*	L	60.6	16.8	4.6	51.8	28.7	12.2	13.6
White Poplar	T	50.4	4.6	1.9	68.7	56.5	25.2	7.0
	42:58	49.9	9.7	3.0	61.6	44.8	19.7	9.8
Salix babylonica	L	59.9	19.5	1.5	37.5	24.2	9.2	9.4
Weeping Willow	T	63.3	4.3	2.4	72.7	67.8	20.0	3.5
	48:52	61.7	11.6	2.0	55.9	47.0	14.9	6.3
Salix nigra	L	63.9	18.5	4.4	56.8	34.3	17.0	8.2
Black Willow	T	51.6	7.7	2.7	72.3	58.5	24.0	4.2
	26:74	45.8	10.5	1.9	71.8	47.5	17.7	8.7
Ulmaceae *Ceitis occidentalis*	L	51.8	10.1	1.5	44.5	28.7	8.0	15,6
Hackberry	T	31.9	5.4	2.0	81.4	54.1	21.1	6.3
	26:74	37.1	6.6	1.9	71.8	47.5	17.7	8.7
Vitaceae *Vitis sp.*	L	66.0	19.2	14.2	40.7	25.0	22.4	15.4
Grapevine	T	87.1	9.3	2.2	70.3	59.1	14.4	8.9
	59:41	74.7	15.1	9.2	52.9	39.1	19.1	12.7

[a]L = Leaf; T = Twig; Leaf:Twig proportions (% wet weight) of whole plant sample as offered.
[b]AD-CP = acid detergent crude protein (bound protein); NDF = neutral detergent fiber; ADF = acid detergent fiber; Lig = sulphuric acid lignin.

Most European zoos feed a greater variety of fruits (especially bananas and grapes) and vegetables, compared to North American zoos (note differences in response frequencies in Table2). In Europe, six groups of colobines are fed eggs, compared with only one group in North American zoos. Different types of green produce are fed with equal frequency in Europe and North America, although in North America spinach and kale are preferred, compared with fennel and lettuce in Europe. European zoos feed a greater variety of browse, especially rose, willow, and poplar.

Browse Composition

Water, crude and bound protein, and cell wall constituents of temperate browses plants cultivated as food for captive animals, sampled in July and September 1994 can be found in Tables 3 and 4.

Paired comparisons within species demonstrated that leaves are higher in water, protein, and ash ($P < 0.01$), and lower in all fiber fractions than twigs ($P < 0.05$). AD-CP differences were not significant. Paired comparisons by species showed that only NDF and ADF differed seasonally ($P < 0.01$) with fiber fractions higher later in the growing season. Leaf percentage in September was also significantly lower ($P = 0.01$) compared to July.

Diet Analyses

Diets did not differ among colobine species within zoos; unfortunately, however, diet information detailed enough for reliable nutrient summary calculations (amount actually consumed vs. those offered) was obtained from only seven of responding facilities.

The nutrient composition of representative diets eaten by five colobine species in six European and one North American zoos is compared with literature values of natural foods, and 12 temperate browses fed to these primates in captivity, Table 5.

In general, zoo diets contained much less fiber and higher protein levels than foods consumed by these primates in nature. Temperate browse plants (n = 11spp.; leaves plus twigs) sampled in New York in summer and autumn contained higher fiber, and lower protein levels, than diets fed in this survey, and may approximate the nutrient content of food items selected by free-ranging colobines. Fast growing roses grown in greenhouses, fed primarily in Europe, contained substantially less fiber and higher protein concentrations than other browses offered, and may not be an appropriate substitute for native foods.

Table 5. Comparison of nutrient levels in zoo diets, native foodstuffs, and temperate browses fed to south-east Asian colobines in European and North American zoos

	Zoo diets[a] (n = 7) mean S.D.	Native foods[a] (n = 154) (range)	Temperate browse (n = 11) (range)	Rose leaves (n = 1)
Water, %	75.4 ±2.4	31.7 - 78.3	37.8 - 80.4	73.9
CP, % DM[c]	15.0 ±3.0	5.2 – 21.1[b]	4.8 - 15.1	20.8
NDF, % DM	12.5 ±4.6	43.7 - 66.7	44.0 - 78.9	32.3
ADF, % DM	6.3 ±2.8	30.5 - 52.3[b]	34.3 - 61.0	17.7
Lig, % DM	1.0 ±1.1	14.5 - 28.3	8.8 - 20.2	3.9

[a]Modified from Nijboer et al. (unpublished).
[b]Additional data from Kay and Davies (1994).
[C]CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; Lig = H_2SO_4 Lignin; DM = dry matter.

Discussion

Many diets in this survey were very complex and were probably developed through experience, rather than applied research. One hundred and forty-nine different ingredients indicates that there is no agreement as to what foods should be fed to these primates In European zoos, a greater diversity of foods are offered. Only one-third of responding zoos knew the exact quantities fed or consumed; all zoos fed some quantity of browse, but responded without any quantification or estimates of intake levels. More detailed intake studies are on southeast Asian colobines particularly relative to browse consumption.

Comparison of zoo diets to natural foods can be one method of approaching a more nutritionally appropriate diet (see Table 5). Chemical fraction of the native foods are listed as ranges only, because available studies in the literature do not provide feeding rates such that weighted averages for native diets could be calculated. Many temperate browse plants offered (leaf plus twig fractions) contain higher amounts of NDF (32.3-78.97%), ADF (17.7-61.0%) and lignin (3.9-20.9) than current zoo diets. Primates in zoos consume both leaf and twigs of browse plants (leaf consumption alone was not reported), including the bark (personal observation); however, the proportions of actual leaf:twig intake have not been documented extensively in zoo collections, making nutrient intake difficult (or impossible) to accurately calculate. It is possible that animals in zoos are eating twig fractions to increase fiber content of the overall diet; however, in browses for which only bark is stripped, primates are eating the more nutritious and relatively less fibrous part of the twig. In these cases, bark should be analysed separately.

Quantification of intake in zoos collections (amount as well as plant parts), along with estimates of consumption in the wild, are needed for more detailed assessment.

Nonetheless, important potential nutrient contributions to diets of zoo colobines are apparent from temperate brows plants cultivated for use in captive feeding programs. Leaves provided 10-20% crude protein, regardless of season sampled, while twigs contained about half that level. Browse fiber levels (NDF and ADF) increased in September in both leaves and twigs, but lignin did not. Thus, fiber present could be potentially more fermentable later in the growing season, but remains to be tested. Of the 11 species evaluated, beech contained notably higher fiber concentrations than other browses. Based strictly on chemical composition, this species (and possibly hackberry) would appear least nutritious. Grape vine (not leaves) might also be considered to be or lower nutritive value for southeast Asian colobines, due to the fibrous intestinal blockage [Calle et al., 1994]. The mineral content of two of the species examined – mulberry and white poplar- should be investigated in detail, due to the high ash content of leaves in this report.

Inclusion of these (or other) browses may be useful in duplicating the natural diet composition of Asian colobines, particularly fiber and protein levels. In contrast, rose browse, which is widely utilized in European facilities, is often grown in rapidly under artificial (greenhouse) conditions, and thus is not exposed to the environment of other browse in this report. Clearly the composition of rose browse is more different, compared with native foods or the other temperate browses described here, and should possibly not be considered the best nutritional substitute to native foods.

More extensive analyses of seasonal variability in browse composition, and the occurrence and physiological effects of secondary plant compounds, are needed. Zoos considered in this survey are not situated in tropical or subtropical areas with fresh browse available year-round, thus harvesting followed by freezing

[Koontz et al, 1988] and drying [Walter and Perschke, 1994] is often employed. Additional information would provide a better indication of optimal harvesting periods, and most suitable browse species. In particular, palatability trials correlated with nutritive content of these browses might provide the best insight into feeding management applications.

It must be noted that very high lignin values indicated here for native foods as well as temperate browses (Table 5) include cutin fractions, due to the analytical methods used. Although indigestible (as in lignin), cutin is a wax and has a very different texture than lignin, being soft rather than harsh. Duplication of chemical fractions in captive diets must take into account such factors since they may affect palatability.

Currently, zoo diets do not appear to duplicate the very high fiber levels described in either temperate browses or native foods eaten by these primates. We propose utilizing these data as a baseline for diet development, and suggest feeding trials using locally available browses, and/or that a commercial product (or products) be advanced such that southeast Asian colobine nutritional management may be more standardized across worldwide zoological institutions.

Conclusions

1 There is currently no standardized diet fed to south-east Asian colobines in captivity.
2 European zoos feed a greater diversity of fruits and vegetables compared with North American facilities, and diets offered by zoos contain less fiber (NDF, ADF and lignin) and more protein than natural foods eaten by these primates. Browses comprised a variable but important fraction of zoo diets.
3 Leaves plus twigs of elm, maple, poplar, and willow trees and commercially cultivated roses were most frequently reported as browses used in feeding southeast Asian colobines in northern hemisphere zoos.
4 Temperate brows plants cultivated for zoo feeding programs may provide essential nutrients and duplicate chemical constituents (particular fiber) or natural diet items for colobines. Trees analysed contained fiber and protein levels within ranges reported for native foods; rose browse, used extensively in European facilities, had less fiber and more protein, and may not be as suitable.

5 The leaf:twig ratio of browses analysed decreased in September, compared with July samples; consequently, fiber content (NDF and ADF) was higher later in the growing season. More detailed studies of browse intake and additional information on chemical composition (including potential toxins) would provide a better indication of optimal harvesting periods, and most suitable browse species for zoo feeding programs.

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CHAPTER 4

Chemical composition of Southeast Asian colobines foods

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Expanded abstract

Digestive disturbance have been considered a major health issue among captive colobines (Janssen, 1994; Calle et al., 1995). Inadequate or inappropriate fiber sources may underlie some of these problems. Apart from maintaining normal gastrointestinal function (Van Soest, 1994), one critical issue in husbandry of these species is the necessity of providing a suitable diet for supporting pregastric (Steven, 1988) fermentation for microbial degradation of plant cell wall constituents as an energy source (Bauchop and Martucci, 1968; Bauchop, 1978; Waterman, 1984). Alternatively, suitable microbial populations may provide important detoxification mechanisms for coping with secondary compounds identified in leaves and seeds consumed in nature (Freeland and Janzen, 1974; Hladik, 1977; Oates et al., 1977; Lebreton, 1982; Waterman, 1984). Furthermore, both excessive soluble carbohydrate (Goltenboth, 1976; Waterman, 1984) and protein (Davies et al., 1988) concentrations in diets fed to captive colobines have been implicated in health disorders.

This report summarizes data on the chemical composition of foods eaten by proboscis monkeys (*Nasalis larvatus*), Hose's langurs (*Presbytis hosei*), and the Ghuizhou snubnosed monkey (*Rhinopithecus brelichi*). Feeding observations and sampling of plants eaten follow standardized methodologies as reported by studies of primate foraging (see for example, Bennet and Sebastian, 1988; Yeager, 1989). Plant materials consumed by the proboscis monekeys were collected in Samunsam Wildlife Sanctuary, Sarawak, Malaysia or Tajung Puting National Park (Talimantan Tengah), Indonesia, over a 10-yr period; those eaten by Hose's langurs were collected in Silabukan Forest Reserve and Tabin Wildlife Reserve, Sabah, Malaysia, over an 18-mo period (Mitchell, 1994), and the plant samples eaten by the Guizhou snub-nosed monkey were collected over a 2-yr field study in the Fanjing mountains of south-western China (Bleisch and Xie, 1994).

Leaf (n=92), fruit (n=16), flower (n=15) and seeds (n=31) samples were air- or oven-dried to a constant weight, and water determined in the field before shipment to the Nutrition Laboratory at the Wildlife Conservation Society, Bronx, NY. All samples were ground to pass a 2 mm screen, and analysed according to AOAC methods for animal feeds (1996). Crude protein (CP) values were determined as total nitrogen x 6.25 using a macro-Kjeldahl method with a copper catalyst; acid detergent nitrogen x 6.25 (AD-CP) was evaluated as a measure of unavailable protein (Goering and Van Soest, 1970).

Table 1. Chemical composition of native food plants (mean ± s.d.) eaten by three southeast Asian colobines. All nutrients on a dry matter basis except water in percentage.

Primate Species	Plant Part (n)	Water	CP	AD-CP	NDF	ADF	Lignin	Fat	Field Site[a]
Nasalis larvatus (Proboscis monkey)	Leaves (n=13)	31.7 ± 19.0	11.7 ±5.5	6.1 ±1.9	57.2 ±14.1	46.3 ±13.3	25.8 ±10.0	NA	1
	(n= 20)	NA	9.9 ±3.4	3.4 ±1.8	43.7 ±11.3	30.5 ±12.1	14.5 ±8.8	NA	2
	Fruits (n=10)	78.3 ±1.7	5.2 ±1.6 (n=22)	3.8 ±0.9	66.7 ±15.3	52.3 ±15.1	28.3 ±14.4	7.4 ±6.9	2
	Flowers (n=10)	37.2 ±19.3	10.4 ±4.0 (n=13)	5.6 ±2.3 (n=9)	50.7 ±7.3	41.6 ±19.3	26.7 ±15.8	3.4 ±0.5 (n=3)	1,2
	Seeds	68.4 ±17.7 (n=14)	8.1 ±2.5 (n=22)	3.6 ±2.0 (n=9)	NA	NA	NA	13.0 ±11.2 (n=11)	1,2
Presbytis hosei (Hose's langur)	Leaves (n=10)	NA	16.0 ±4.0	7.4 ±3.7	54.6 ±19.6	42.2 ±19.2	24.4 ±13.1	NA	3
	Flowers (n=2)	NA	10.0 ±3.4	7.4 ±3.7	54.6 ±19.6	42.2 ±19.2	24.4 ±13.1	NA	3
	Seeds (n=9)	NA	10.9 ±4.7	5.8 ±3.8	NA	NA	NA	17.0 ±15.2	3
Rhinopithecus brelichi (Ghuizhou snub-nosed monkey)	Leaves (n=49)	NA	14.0 ±4.1	5.3 ±2.4	47.8 ±12.4	37.8 ±12.8	19.5 ±8.1	NA	4
	Fruits (n=5)	NA	5.7 ±2.1	1.5 ±1.2	51.3 (n=1)	40.6 (n=1)	19.9 (n=1)	11.4 ±11.9	4

CP= crude protein; AD-CP = bound protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NA = not analyzed
[a] 1 = Bennet and Sebastian, 1988; 2 = Yeager, 1989; 3 = Mitchell, 1994; 4 = Bleisch and Xie, 1994

Neutral detergent fiber (NDF), acid detergent fiber (ADF), and sulphuric acid lignin values were quantified using the methods of Goering and van Soest (1970) with no pretreatments or enzymes. Crude fat extractions (fruits, flowers, and seeds only) were performed using petroleum ether. Total ash content was obtained by heating sample to 550^{o} C. overnight in a muffle furnace. Mineral concentration were determined in triplicate by atomic absorption spectroscopy after dry ash digestion (Perkin Elmer, 1982). Proximate and mineral data are reported in Tables 1 and 2.

Table 2. Mineral composition of native food plants (mean ± s.d.) eaten by three southeast Asian colobines, compared with recommended dietary levels for nonhuman primates (NRC, 1978).All nutrients on a dry matter basis.

Primate Species	Plant Part (n)	Ash %	Ca %	Mg %	Cu mg	Fe mg	Mn Mg	Zn mg	Field Site[a]
Nasalis larvatus (Proboscis monkey)	Leaves (n=23)	6.3 ±2.8	0.3 ±0.3	0.2 ±0.1	±7.2 ±6.6	43.9 ±22.9	26.8 ±31.8	10.9 ±4.9	2
	Fruits (n=17)	3.5 ±1.4	0.5 ±0.3	0.3 ±0.1	7.0 ±4.1	29.5 ±18.1	127.0 ±176.2	10.8 ±6.4	2
	Flowers (n=6)	5.7 ±2.7	0.5 ±0.3	0.3 ±0.1	9.0 ±3.6	33.6 ±12.8	20.9 ±18.9	18.9 ±11.4	1,2
	Seeds (n=19)	3.3 ±1.2	0.2 ±0.3	0.2 ±0.1	9.0 ±3.6	33.6 ±12.8	20.9 ±18.9	18.9 ±11.4	1,2
Presbytis hosei (Hose's langur)	Leaves (n=3)	7.8 ±4.9 (n=10)	0.4 ±0.1	0.3 ±0.1	13.0 ±3.7	70.4 ±30.0	579.5 ±535.9	31.0 ±12.0	3
	Seeds (n=8)	7.9 ±12.2	0.5 ±0.7	0.1 ±0.1	10.3 ±3.0	65.6 ±50.4	174.4 ±150.7	28.7 ±28.4	3
Rhinopithecus brelichi (Ghuizhou snub-nosed monkey)	Leaves (n=7)	8.3 ±3.5 (=49)	1.6 ±0.5 (n=6)	0.3 ±0.1	9.1 ±3.4	154.5 ±48.0	708.5 ±817.8	58.2 ±59.2	4
Non-human primate recommendations		None	0.54	0.16	None	196	None	11	

[a] 1 = Bennet and Sebastian, 1988; 2 = Yeager, 1989; 3 = Mitchell, 1994; 4 = Bleisch and Xie, 1994

For all three species, chemical composition of plants was included in average if the plant occurred in the diet. No weighting of foods (i.e. by percent of diet) was attempted for this broad summary. The food choices of Asian colobines vary seasonally in the wild. When available, fruits and seeds are a frequent component of the diet; during times of low fruit availability, these primates typically switch to eating leaves (Yeager, 1989; Kool, 1993). Nonetheless results of this survey suggest chemical similarities among food items.

Overall, protein content in foods eaten by these primates averaged 12.9 ± 4.4% of dry matter, of which almost 50% analysed as AD-CP. or nutritionally unavailable, protein. Both protein comprised 31-52% of crude protein in leaves, 54-84% in flowers, and 44-53% in seeds. While crude protein levels are identical, available protein (CP less AD-CP) in native plants appears considerably lower than the concentration recommended by the National Research Council (NRC, 1978) for feeing nonhuman (and noncolobine) primates (16.3% of dietary dry matter), as well as those reported in a survey of diets fed to colobines (15±3.0) in European and North American zoos (Nijboer and Dierenfeld, 1996). Available protein levels measured in preferred plants approached minimums required for the maintenance of microbial populations in ruminant herbivores (Van Soest, 1994), and ruminant nutrient requirements may better model the dietary protein needs of colobine monkeys, but has not been investigated in detail.

All food categories were highly fibrous, with even leaves containing >50% NDF. The degree of lignification (Lignin/NDf) in leaves (41-45%) did not differ from the measured in fruits (37-42%; n=16); lignification in flowers averaged 52±2% (n=12). These values contrast markedly with low fiber (12.5 ±4.6%), and very low lignin (1.0±1.1%)diets evaluated for zoo colobines (Nijboer and Dierenfeld, 1996). Physical as well as chemical components of fibrous constituents of herbivore diets are known to influence gut motility and function, as well as play an important role in fecal integrity (Van Soest, 1994), and may underlie current health problems noted for these species in captivity. Temperate browses fed in many zoos appear to provide suitable chemical substitutes for native browses for some of these species, but effects on digestive physiology have not yet been quantified in controlled studies.

Seeds and fruits would appear to be a variable source of dietary fat for colobines, possibly providing significant energy, but effects of dietary fats upon either colobine monkeys, or their gastrointestinal microbes, have not been investigated. Regarding the mineral content of plant evaluated in this study, both Ca and Fe may be limiting compared with primate recommendations; again, ruminant dietary requirements may provide a more suitable model. Calcium in flowers and seeds eaten by langurs and proboscis monkeys was higher than in many leaf samples. This finding was unexpected, as cultivated seeds and nuts are known to contain low Ca content as rule, whereas leaves are often considered a primary Ca source.

Implications for captive feeding programs

Although wild proboscis monkeys and langurs tend to select food higher in protein and lower in fiber than non preferred plant species (Yeager, 1989; Kool, 1993; Yeager et al., 1997), the natural foods eaten by these primates are not high in protein, and contain much higher levels of indigestible fiber than most zoo dietary ingredients. The lack of mineral data for most browses (either native or those substitutes fed in zoos) limits their assessment as feeds, and needs to be addressed. Detailed analysis and comparison of natural feedstuffs may provide useful information for the development of optimal diets for managed populations of colobines.

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CHAPTER 5

Chemical analysis and consistency of faeces produced by captive monkeys (François langurs, *Trachypithecus françoisi*) fed supplemental fibre

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Abstract

The effect of additional dietary fibre on the consistency of faeces was studied in a group of four François langurs (*Trachypithecus françoisi)* kept in Rotterdam Zoo. To increase fibre intake, a diet pellet rich in fibre was offered instead of the usual, commercial primate pellet. This dietary change raised the amounts of hemicellulose and cellulose that were consumed at the expense of non-structural carbohydrates. The experiment had an A1-B-A2 design. Stool quality improved when the high-fibre pellet was fed. The monkeys produced somewhat more faecal dry matter and the faeces contained markedly more non-structural carbohydrates and less crude fibres when the high-fibre pellet was fed. The percentage of water in the faeces was slightly lower when the high-fibre diet was offered. We speculate that the extra fibre was partly fermented and that the breakdown products were recovered in the carbohydrate fraction of faeces. These breakdown products might have a superior water-binding capacity, leading to well-shaped faeces. This study showed that François langurs have the capacity to digest dietary fibre, as has been demonstrated earlier for other species of leaf-eating monkeys.

Key words: dietary fibre; digestion; leaf-eater monkeys.

Introduction

The François langur (*Trachypithecus françoisi*) is a herbivorous primate that inhabits the sandstone hills in the south of China and north of Vietnam. The natural diet of this primate mainly consists of various types of leaves, but fruits are also consumed (5). François langurs and other leaf-eating primates of the subfamily Colobinae have a stomach consisting of different compartments for pregastric fermentation similar to that in ruminants (1, 4). However, langurs do not ruminate (1,2,4).

Like many other langur species, the François langur is threatened by extinction both in the wild (5) and in captivity (6). To increase the survival of these species in captivity, proper dietary management is needed. Unfortunately, there is only limited information in this respect. Leaf-eating monkeys in captivity often suffer from gastrointestinal disturbances associated with diarrhoea (3). Comparison of the natural diet of leaf-eating monkeys and Western zoo diets has shown that the former contains much higher levels of fibre (6). Because of this and because the François langurs in Rotterdam Zoo produced soft faeces, a diet pellet rich in fibre was introduced to their diet.

The experimental high-fibre pellet was offered instead of the usual, commercial primate pellet. This paper reports the effect of extra fibre intake on the composition and consistency of faeces produced by the langurs.

Methods

Four François langurs were studied. There were two males aged 11 and 13 years, one female aged 13 years and one juvenile female aged 3 years. The langurs were housed together in an enclosure consisting of an indoor (5.00 x 3.50 x 3.65 m) and outdoor area (15.50 x 3.50 x 3.65 m). The outdoor enclosure had natural daylight. Lights in the indoor enclosure were on from 7.00 to 19.00 hours. The experiment was conducted between the end of May and the beginning of August 1999. The experiment had an A1-B-A2-design. The usual diet was fed during the first period (A1), the high-fibre diet was fed during the second period (B), and the usual diet was reintroduced for the last period (A2). Periods A1 and B lasted 21 days and the A2 period lasted 24 days. During the first week of periods B and A2, the pellets to be fed were gradually introduced.

Table 1. Average composition of the diet (g/kg as fed) offered to the four langurs during each period of the experiment.

Period Days	A1 (n=7)	B (n=7)	A2 (n=7)
Pellets[1]	68.6	152.6	70.9
Greens (lettuce, cucumber, fennel, celery)	176.3	224.9	154.6
Browse (willow branches, rose leaves)	557.5	458.3	576.2
Vegetables (carrot, green pepper, beetroot)	153.2	126.1	146.5
Fruits (green banana)	34.9	28.2	44.8
Egg (boiled whole)	7.4	7.6	4.9
Leaf-eater vitamins[2]	2.1	2.3	2.1
Total	1000	1000	1000

[1]Period A1 and A2 : Leaf-eater Pellets, Mazuri Zoo Foods, Witham, Essex, U.K. Composition per 100 g: moisture 10 g, crude protein 23 g, crude fat 5 g, crude fibre 11.2 g, NDF 23.7 g, ADF 15 g, calcium 1030 mg, phosphorus 670 mg, sodium 270 mg, potassium 106 mg, magnesium 210 mg, iron 39 mg, copper 2.5 mg, zinc 14.5 mg, manganese 12.5 mg, vitamin A 3000 IU, vitamin D3 660 IU, vitamin E 21mg, vitamin C 100 mg.
Period B: Experimental pellet produced by Hope Farms, Woerden, The Netherlands. Composition per 100 g: moisture 4 g, crude protein 19.8 g, crude fat 6.3 g, crude fibre 52.1 g, NDF 52.8 g, ADF 39 g, lignin 8 g, calcium 980 mg, phosphorus 420 mg, sodium 32 mg, potassium 630 mg, magnesium 48 mg, iron 35.4 mg, copper 4.2 mg, zinc 11.9 mg, manganese 13.2 mg, vitamin A 2183 IU, vitamin D3 437 IU, vitamin E 12.9 mg, vitamin C 18.3 mg.

[2]Leaf-eater vitamins per 10 g (38.7% Mervit sporavit 325, 60.5% calcium monophosphate, 0.8% vitamin: Pre Mervo 500): moisture 0.2 g, ash 5.9 g , vitamin A 41401 IU, vitamin D3 774.3 IU, vitamin E 11.6 mg, thiamine 1.6 mg, riboflavin 3.1 mg, niacin 15.5 mg, pyridoxine 1.6 mg, folic acid 774.3 µg, vitamin B12 11.6 µg, pantothenic acid 7.7 mg, choline 154.9 mg, biotin 0.04 mg, vitamin C 38.7 mg, calcium 1427.8 mg, phosphorus 1461.7 mg, magnesium 35.7 mg, potassium 4.8 mg, sodium 3.6 mg, sulphur 72 mg, iron 139.1 mg, zinc 37.3 mg, copper 3.9 mg, manganese 40.8 mg, selenium 0.08 mg, iodine 0.15 mg, cobalt 0.22 mg.

Then, the diet was kept constant, and diet and faeces samples were collected during the last 7 days of each period. Diets offered are shown in Table 1. At 8.00 hours, the monkeys were offered the diet pellets. At about 10.00 hours, they were given half of the remaining daily food, and the other half was offered at 16.00 hours.

During the last 7 days of each period, stool quality was scored daily on a scale of 1-5. The scores were defined as follows:

1 = properly shaped and generally solid,
2 = properly shaped, but soft,
3 = in part properly formed,

4 = loose faeces,
5 = liquid faeces.

As a reference for properly shaped faeces, we took the form produced by wild François langurs, as shown in a paper of Nadler (5). Scores were assigned by F.B. Duplicate samples of the diet and left-overs were collected daily for the last 7 days of each period. Faeces were collected quantitatively. All items collected were stored at – 20°C until analysis. The diet and faeces samples were dried at 60°C, pooled per period, and then ground to pass through a 1-mm screen. All samples were analysed for dry matter, nitrogen, crude fat, crude fibre, and ash using the Weende method. Crude protein was calculated as nitrogen mass times 6.25. Non-structural carbohydrate content was calculated as the residual fraction. Further, neutral- (NDF) and acid-detergent fibre (ADF) and lignin were analysed using the method described by Van Soest (9). Hemicellose was calculated as NDF minus ADF and cellulose as ADF minus lignin.

Results

Table 2 shows the amounts of macronutrients supplied, left-over and consumed. The feeding of the experimental pellet during period B raised the amounts of crude fibre, NDF and ADF in the diet that was actually consumed by the monkeys (Table 3). The amount of cellulose ingested was also increased. During period B, the concentration of non-structural carbohydrates in the diet consumed was lower than during periods A1 and A2 (Table 3).

Thus, in essence the intake of fibre during period B was increased at the expense of non-structural carbohydrates. Replacing the usual diet pellets by the high-fibre pellets did not systematically alter the amounts of other nutrients consumed. The langurs were reluctant to consume the high-fibre pellet in the beginning, but they readily consumed it, although slower, when the amount of the usual pellet was reduced and finally removed from the diet.

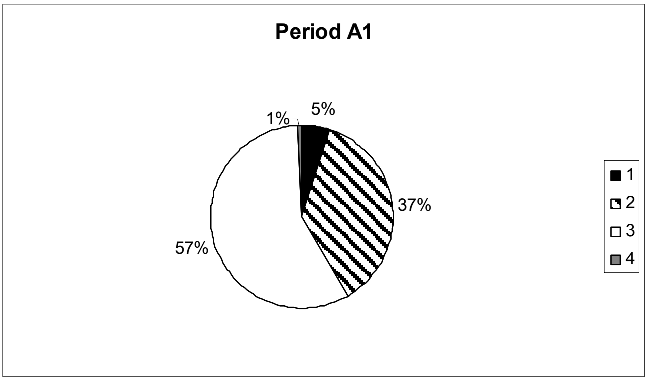
Table 2. Amounts of macronutrients supplied, left-over and consumed and that recovered in faeces (g/animal day)

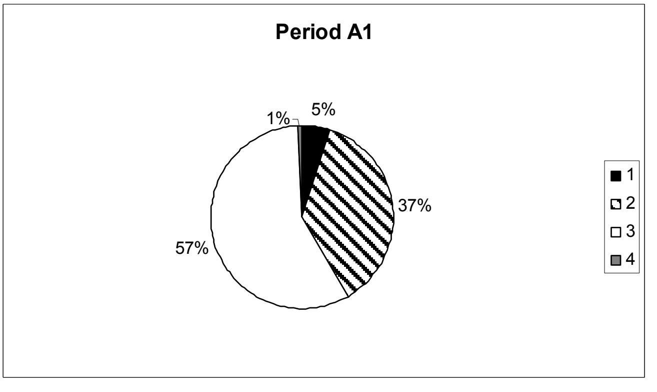
	Supplied			Left-over			Consumed			Faeces		
Period:	A1	B	A2	A1	B	A2	A1	B	A2	A1	B	A2
Protein	53	69	54	26	31	17	28	38	37	14	12	11
Fat	12	16	14	6	7	4	6	9	9	5	7	5
Carbohydrates	178	162	166	107	87	75	70	75	92	17	38	14
Crude fibre	115	142	107	90	88	81	25	54	26	14	2	10
NDF	202	242	193	144	144	119	58	98	75	31	37	24
Hemicellulose	50	72	54	31	40	28	18	32	26	7	10	7
ADF	153	170	140	113	103	91	39	66	49	24	29	17
Cellulose	91	114	93	71	70	60	19	44	33	8	16	8
Lignin	62	55	47	42	33	31	20	22	16	15	14	9
Ash	24	27	28	12	12	11	12	14	17	8	8	7
Water	974	857	1011	253	222	300	721	636	711	196	182	158
Dry matter	382	416	369	241	225	188	141	190	181	58	67	47

The water fraction of the macronutrients supplied and consumed does not include drinking water

Table 3. Composition of the diets (g/kg dry matter) consumed by the four François langurs.

Period:	A1	B	A2
Protein	199	200	204
Fat	43	47	50
Carbohydrates	496	395	508
Crude fibre	177	284	144
NDF	411	516	414
Hemicellulose	128	168	144
ADF	277	347	271
Cellulose	135	232	182
Lignin	142	116	88
Ash	85	74	94





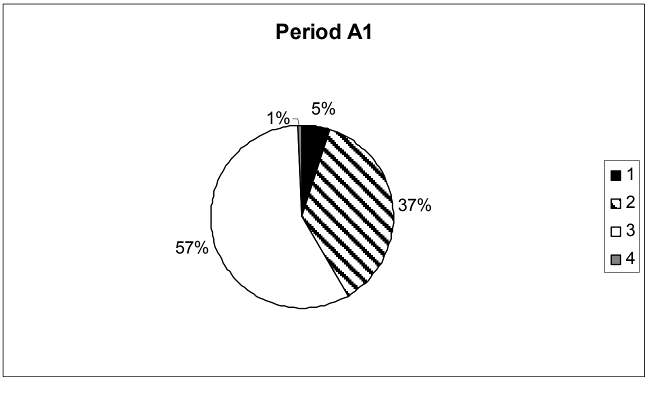


Figure 1. Proportional distribution of stool quality in periods A1 (control diet), B (high-fibre diet) and A2 (control diet). Indicated areas correspond with faecal scores: 1, properly shaped and generally solid, 2 properly shaped, but soft, 3, in part properly formed, 4, loose faeces.
The latter score was given for 1% of the faeces during period A1 only.

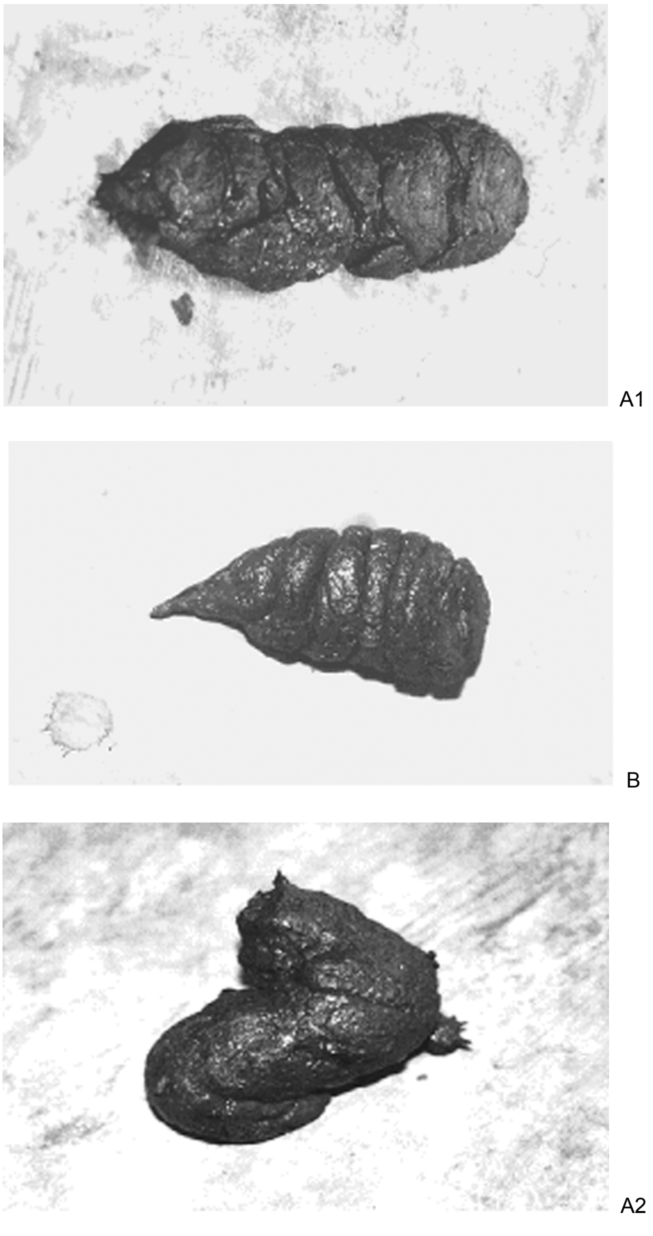
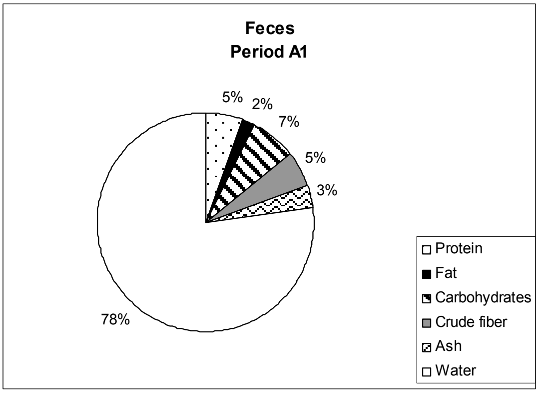
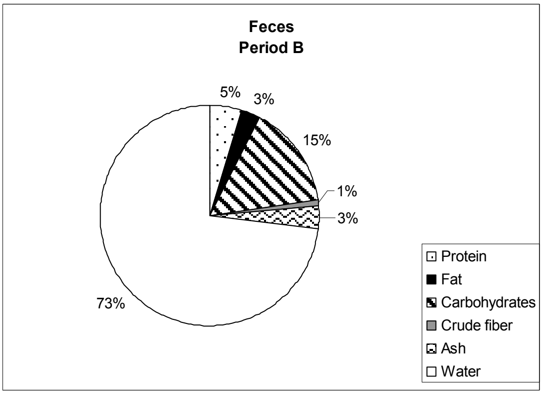


Figure 2. Typical appearance of faeces produced during the A1 (control diet), B (high-fibre diet) and A2 (control diet) periods.





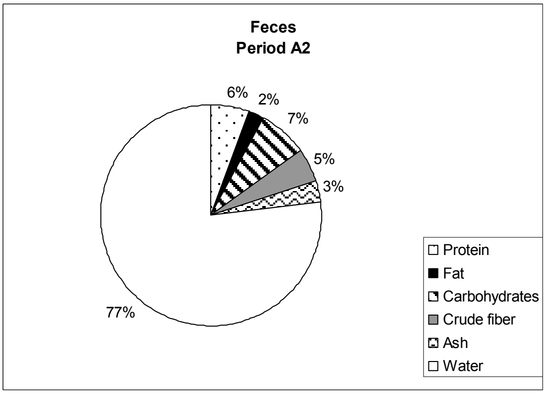


Figure 3. Proportional composition of the faeces in periods A1 (control diet), B (high-fibre diet) and A2 (control diet). Explanation of different areas:

The scores from stool quality are given in Figure 1. Stools were more properly shaped and solid when the langurs were fed the high-fibre diet. The stools were less well-shaped and solid when the usual diet was offered during either period A1 or A2.

Photographs of typical faeces also show the difference in stool quality between the B and the two A periods (Figure 2). Table 2 shows the chemical composition and amount of faeces produced.

During period B, the monkeys produced somewhat more faecal dry matter than during the A periods. The amount of faecal water was not systematically affected by the diet (Table 2), but the percentage of water in faeces was slightly lower during the B period (Figure 3). When monkeys ate the high-fibre pellets, the faeces contained more non-structural carbohydrates (15 versus 7%) and less crude fibre (1 versus 5%) than when they ate the usual pellets (Figure 3).

The data in Table 2 allow calculation of the apparent digestibility of macronutrients. Apparent digestibility is defined as nutrient intake minus faecal excretion and expressed as a percentage of intake. However, for digestibility calculations the amount of macronutrients consumed may not be sufficiently accurate because they reflect the difference between two relatively large values, i.e. the amount offered and that left over. The major portion of the left-overs contained branches from which the leaves had been removed. As a consequence, in a few cases the apparent digestibility of macronutrients, calculated as the amount consumed minus the amount recovered in faeces, may show aberrant values. The apparent digestibility of crude fibre was 96% for the experimental diet and 46% (period A1) of 62% (period A2) for the usual diet.

Discussion

From the outset, it should be stressed that his study had various limitations which relate to the use of rare, exotic animals on exhibit in a zoo. The four langurs studied were housed in a group so that the inter-individual variation in digestibility measurements could not be assessed. In addition, the animals were of different age and body weight. The provision of different food items as a mixed diet allowed for selection and thus inter-individual variation in intake. The diets consumed during the two A periods were similar, but not identical. It cannot be excluded that there was some urine contamination of faeces. Thus, the group mean data on faeces composition should be interpreted with caution, but it is reassuring that the reproducibility was acceptable, which would exclude any substantial difference in systematic and/or random error for the two A periods.

This study showed an improvement of stool quality when the François langurs were fed a high-fibre diet. The appearance of the stools during period B was similar to that observed when François langurs are in their natural habitat (5). As shown in table 3, the experimental high-fibre diet, as consumed during period B, contained 28% crude fibre, 52% NDF, 35% ADF and 12% lignin in the dry matter. In this zoo, the usual diet as consumed during periods A1 and A2, on average contained 16% crude fibre, 41% NDF, 27% ADF, and 12% lignin in the dry matter (Table 3). Diets consumed by south-east Asian colobines in nature may contain 51% NDF in the dry matter whereas diets fed to leaf-eating monkeys in Western zoos contain only 4-26% NDF (6). Thus, the high-fibre diet used in this study corresponds with the diet that may be consumed in the wild, at least with regard to the concentration of NDF.

The leaf-eating monkeys such as langurs (1, 8), proboscis monkeys (2) and colobus monkeys (7, 10) can efficiently utilise cell wall constituents. Indeed, in this study the apparent digestibility of hemicellulose and cellulose ranged between 62-73% and 57-77% of intake, respectively. Leaf-eating monkeys are often fed the same diet as are the more frugivorous monkeys and apes, which may give rise to gastrointestinal disturbances in the former (3). To maintain proper gastrointestinal function in leaf-eating monkeys the diet may have to contain high amounts of fibre. This statement is supported by the present observation in that replacement of non-structural carbohydrates by hemicellulose plus cellulose improved stool quality in François langurs.

It is difficult to see why the experimental high-fibre diet improved faecal consistency. With the experimental diet, the faeces contained slightly less water, were markedly enriched with no-structural carbohydrates, and had a lower content of crude fibre. Crude fibre consumption was about twice as high when the experimental diet was fed instead of the control diet, whereas the absolute amount of non-structural carbohydrates ingested was similar for the two diets. Possibly, the crude fibre was partly fermented so that its breakdown products were recovered in the carbohydrate fraction of faeces. The breakdown products of crude fibre might have a superior water-binding capacity, leading to well-shaped faeces. Further research could prove or disprove these ideas. In keeping with the idea of partial digestion of crude fibre, the apparent digestibility of the crude fibre was much better when the high-fibre diet was offered instead of the control diet. Our data point to an apparent digestibility of lignin of about 35 %. Although it is generally accepted that lignin can neither be digested nor fermented some authors have demonstrated that lignin apparently can be digested by leaf-eating monkeys (2, 7, 10). However, the observed digestibility of lignin has been ascribed to methodological error (9), as described by Watkins *et al.* (10). Despite this, it cannot be excluded that leaf-eating monkeys have intestinal bacteria capable of breaking down lignin.

In summary, this study shows an improvement of stool quality when François langurs were fed a high-fibre diet. However, long-term feeding trials will be necessary to determine whether a high-fibre diet can enhance the health and reproductive success in François langurs and other leaf-eating monkeys kept in captivity. This study also showed that although the langurs preferred a diet containing carbohydrates that are easily digested, they also consumed a diet containing more fibre albeit less willingly and slower.

Acknowledgements

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CHAPTER 6

THE INFLUENCE OF INTRODUCING A HIGH-FIBER PELLET ON THE TIME BUDGET OF CAPTIVE FRANCOIS LANGURS (Trachypithecus françoisi)

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Abstract

In a group of four captive François langurs at Rotterdam Zoo, The Netherlands, a high-fiber pellet was introduced. To see if this change in diet would influence the time budget of the François langurs, the behaviour was studied before and after the introduction. As a control the high-fiber pellet was removed again and the behaviour was studied once more. The events that were studied were feeding, resting, moving, grooming and aggressive behaviour.

The time budgets did not vary significantly between the three periods of observation (feeding on average 20%, resting on average 62%, moving on average 10%, grooming on average 10% and aggressive behaviour on average 0.33%). Further the average time budget of captive François langurs was similar to what has been observed in wild white-headed leaf monkeys in China.

A closer look at the daily distribution of time spent on feeding showed that the introduction of the high-fiber pellet resulted in less distinct feeding peaks, and feeding was distributed more evenly over the whole day. This also corresponds well with what has been observed in wild white-headed leaf monkeys.

Introduction

François langurs (*Trachypithecus françoisi*) are restricted to small areas in south China and North Vietnam. The species is endangered both in the wild (Nadler, 1994) and in captivity (Nijboer et al., 1996).

François langurs are herbivorous and have stomachs with different compartments where pregastric fermentation occurs similar to that in ruminants. However, langurs do not ruminate (Kuhn, 1964, Bauchop and Martucci, 1968, Dierenfeld and Koontz, 1992).

In captivity langurs often suffer from gastrointestinal disturbance associated with diarrhoea (Hill, 1964). To improve their health, maintenance and reproduction the emphasis has been laid on providing a suitable diet to support the pregastric fermentation. A comparison between natural diets and diets fed in captivity has shown that the former contain higher quantities of fiber (Nijboer et al., 1996).

In Rotterdam Zoo a high-fiber pellet was introduced in the diet of a group of François langurs (Nijboer et al., 2001). Because changes in the diet may influence the behaviour, the behaviour was monitored. So far, little field study has been reported on the François langur, and natural behaviour data are not known. This paper reports the effect of the high-fiber diet on the time budgets of the François langurs in captivity.

Methods

Subjects

The data concern a group of François langurs from Rotterdam Zoo (Nijboer et al., 2001). The group consisted of two males aged 11 and 13 years, one female aged 13 and a juvenile female aged 3 years (born in captivity in Rotterdam Zoo). The group was housed in an indoor enclosure (5.00 x 3.50 x 3.65 m) and an outdoor enclosure (15.50 x 3.50 x 3.65 m). The outdoor enclosure had natural daylight. In the indoor enclosure artificial light was turned on from 07:00 to 19:00 hours. Every day the langurs were fed pellets at 08:00 hours. The enclosures were cleaned between 08:00 – 10:00 hours. At around 11:00 hours one portion of the remaining food (vegetables and leaves) was fed and the second portion at around 16:00 hours. In all three periods the diet consisted of pellets, greens (lettuce, cucumber, fennel, celery), browse (*Salix sp*., *Rosa sp*.), vegetables (carrot, green pepper, beetroot), fruit (green banana), egg (one egg per individual once a week) and leaf-eater vitamins. In periods A_1 and A_2 the Mazuri Old World Monkey Pellet was offered while in period B a high fiber pellet from Hope Farms, Woerden, The Netherlands, was offered.

Data collection and analysis

During the periods in which the feeding trial took place, the behaviour was recorded. In period A_1 and B the behaviour was recorded for 9 days a total of 1414 and 1402 minutes respectively. In period A_2 the behaviour was recorded for 6 days a total of 1245 minutes. The observation took place between the end of May and the beginning of August 1999. The recordings were spread evenly between 10:30 (after cleaning the cages) and 16:30 hours.

The behaviour of the group was recorded using instantaneous time sampling (scan sampling) (Altman, 1974). The sample interval lasted for one minute during which the behaviour of all individuals in the group was recorded simultaneously. The behaviour that was recorded was feeding (F), resting (R = sitting, lying, sleeping), moving/locomotion (M), aggressive behaviour (AG = lunge, hit, bite, chase), grooming (GR), and other behaviour that did not come into one of these categories (O).

Table 1: overview category and time schedule used during the A and B periods.

Category	Time
1	(10:30-10:59)
2	(11:00-11:29)
3	(11:30-11:59)
4	(12:00-12:29)
5	(12:30-12:59)
6	(13:00-13:29)
7	(13:30-13:59)
8	(14:00-14:29)
9	(14:30-14:59)
10	(15:00-15:29)
11	(15:30-15:59)
12	(16:00-16:29)

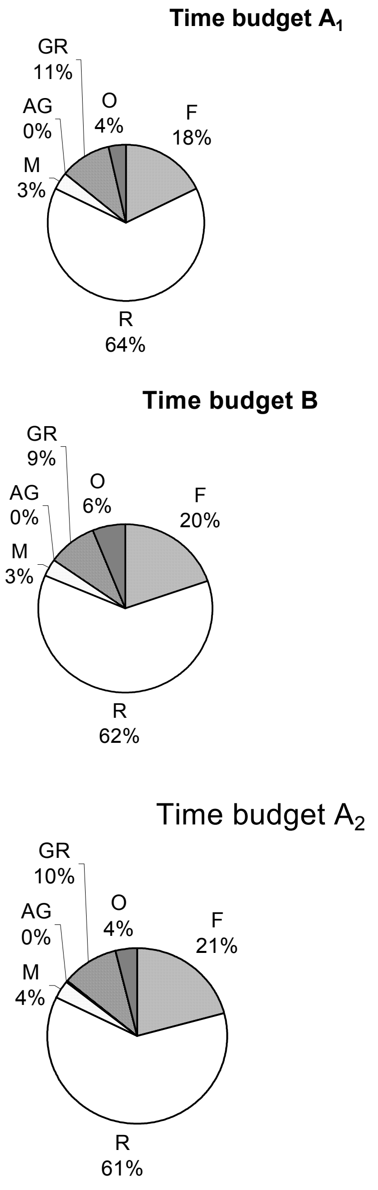

Figure 1: Time budgets in period A_1 and A_2 (fed control diet) and B (fed high-fiber diet). F = feeding; R = resting; M = moving; AG = aggressive behaviour; GR = grooming; O = other

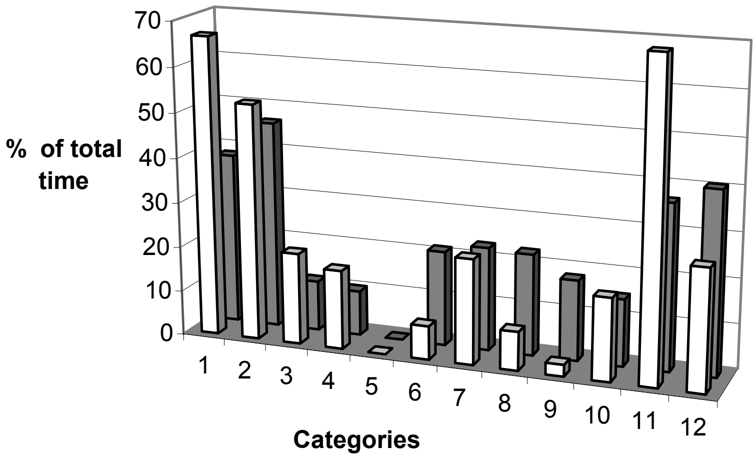

Figure 2: Distribution of time spent feeding. The whit bars are the average of period A_1 and A_2, the black period is the average of period B. Each category lasts 30 minutes.

For each behaviour the frequency was calculated, taking the average of the frequencies of the four individuals in one period. The frequency was converted into a percentage of the total amount of behavioural events. The Chi-square test for k independent samples was used to see if there were any significant differences in the behavioural events between the three periods. The event feeding was further analysed for the distribution in time to see if there was any pattern when feeding occurred during an average day. For the periods A and B the data were divided into 12 time categories (Table 1). For each period the frequency of feeding in each category was calculated taking the average frequency of four individuals in all 12 categories. The Chi-square test for k independent samples was used to test if there was any significant difference between period A_1 and B.

Results

The results of the time budgets from the three periods are presented in Figure 1. There is no significant difference in the time budgets between the tree periods (χ^2= 0,02; df= 10). A closer look at the time spent on feeding distributed over the total observation time is represented in Figure 2. The two periods differ significantly in the distribution of feeding time throughout the observation time (χ^2= 80,34; df= 20). Both periods have clear peaks around feeding time in which the main behaviour is feeding (in category 1, 2 and 11). In period B however, the time spent on feeding is spread more evenly over the 12 categories than in period A1. No observations were made between 12:30 –13:00 (category 5).

Discussion

The evaluation of the time budgets in the three periods showed that there was no significant difference between them. This indicates that the change in the diet does not have any effect on the behaviour of the four langurs. Because so little is known about the behaviour of wild François langurs, it is not possible to tell if the time budgets are representative for the species normal behaviour.

However, data from observations of wild white-headed leaf monkeys (*Trachypithecus françoisi leucocephalus*) (Huang et al., 1998) could be used to compare the time budgets of the captive François langurs. It has been argued that the white-headed leaf monkey is a subspecies of the François langur (also called black langur). Similar to the François langurs the white-headed leaf monkeys also inhabit the sandstone hills of South China. The wild white-headed leaf monkeys spent some more time moving (on average 7.85%) than the captive François langurs (on average 3%). In the captive François langurs somewhat more time was spent on feeding (on average 20%) than was observed in wild white-headed leaf monkeys (on average 14.87%). Further, the time spent resting in the captive François langurs (on average 62%) was almost similar to that observed in wild white-headed leaf monkeys (69.89%).

The fact that the captive François langurs spent less time moving is most likely the result of the limited space they have in the zoo. In both wild and captive animals the time spent resting makes up the major part of the time budget and is characteristic for leaf-eating monkeys because leaf eaters need more time to digest the food rich on fiber (Clutton-Brock et al. 1977).

Although the introduction of the new pellet did not have a significant effect on the time budget of the François langurs, only long-term study can show if this introduction may have positive effects on the health and reproductive success of the François langurs in Rotterdam Zoo.

The results in Figure 2 show clear peaks around feeding times in both period A1 and B but the introduction of more less easy digestible carbohydrates (period B) results in a more even distribution of the time spent on feeding throughout the day. Although not recorded the François langurs clearly preferred the old pellet and consumed it faster than the new high-fiber pellet. As a consequence the old pellet would be finished faster and usually little was left over shortly after offering the pellet. The new high-fiber pellet was not consumed immediately after offering and some would be left for consumption later, which resulted in the more evenly distribution of time spent on feeding. Although feeding peaks have also been observed in wild white-headed leaf monkeys (Huang, 1998) here the feeding still continues during the day in the same manner as was observed in the captive François langurs in period B (Figure 2).

The conclusions of this study are first of all that the introduction of additional less easy digestible carbohydrates to the diet of the four François langurs had no significant effect on the langurs time budget. Further, the time budgets observed corresponded well with what has been observed in wild white-headed leaf monkeys. Secondly , the introduction had a positive effect on the daily rhythm of feeding. It seems as if the results in period B correspond better with what has been observed in wild white-headed leaf monkeys.

Acknowledgement

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CHAPTER 7

Macronutrient digestibility and faeces quality in captive black and white colobus (*Colobus guereza*) and captive spectacled leaf monkeys (*Trachypithecus obscurus*)

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Abstract

Digestibility experiments were carried out with colobus monkeys (*Colobus Guereza*) and spectacled leaf monkeys (*Trachypithecus obscurus*) in an attempt to relate diet composition, apparent macro nutrient digestibility and faeces quality. The spectacled leaf monkeys were fed diets with different amounts of crude fibre, NDF and ADF. Faeces produced by the colobus monkeys were considered as well shaped and generally solid. The spectacled leaf monkeys, which were fed diets with lower fibre content than the diet of the colobus monkeys, generally produced soft and shapeless faeces and sometimes even watery diarrhoea. An increase in the amount of crude fibre and cellulose in the diet of the spectacled leaf monkeys resulted in an improvement of the faeces quality.

For the three diets and the two monkey species studied, it would appear that high intakes of crude fibre and cellulose correlate with high amounts of crude fibre plus carbohydrates (nitrogen-free extract) in the faeces. Apparent digestibility of crude fibre in the colobus and leaf monkeys was as high as 52 – 68 % of the intake. This study indicates that a high intake of crude fibre, including cellulose may improve stool quality in spectacled leaf monkeys.

Key words

Colobines, macronutrients, digestibility, faeces quality, Colobus, *Guereza*, black and white colobus, *Trachypithecus obscurus*, spectacled leaf monkey

Introduction

The colobines comprise so-called African and Asian colobines. Colobines have a tripartite stomach that is divided into a *saccus gastricus*, a *tubus gastricus* and a *pars pylorica*. The stomach has the capacity to ferment cellulose-based foods (BAUCHOP & MARTUCCI 1968, CATON 1999). The proximal colon also is an important site of fermentation (CATON 1999).

The colobus monkeys (*C. guereza*) can be found in Central Africa, between Senegal and Zanzibar, and in areas between the Ethiopian highlands and the southern edge of the Congo basin. They flourish in forest habitats in the savannah zone and in the primary forests of Central Africa. The habitual diet of the colobus monkey in the Kibale Forest consists of 65 % (weight %) immature foliage, including leaf buds, 13 % mature foliage, 3 % foliage of undetermined age, 2 % flowers and floral buds, 15 % fruits, including seeds, and 2 % other plant material, including stem and bark (OATES 1994).

The spectacled leaf monkey (*Trachypithecus obscurus*) also is a herbivorous primate. Its natural habitat includes Sri Lanka, South and Eastern India, Bangladesh, Burma, Indo China, the Malaysian Peninsula and the Sunda Islands. Their natural diet at the Krau Game Reserve in West Malaysia, according to Curtin (1976), consists of 36 % young leaves, 22 % mature leaves, 7 % flowers, 32 % fruits, and 3% seeds. According to Hardy (1990), the natural diet of the spectacled leaf monkey consists of 49 % leaves, 13 % flowers, 26 % fruits, 10 % seeds and 2 % other plant material. Spectacled leaf monkeys are mainly folivorous throughout the seasons (CURTIN 1976, 1980).

Captive colobus and spectacled leaf monkeys are offered diets that can differ considerably from their natural diets. The goal of this study was to compare and contrast macronutrient digestibility and consistency of faeces in a troop of colobus monkeys kept at Rotterdam Zoo and spectacled leaf monkeys kept at Artis Zoo, The Netherlands. The spectacled leaf monkeys received a diet either low (diet B) or high in fibre (diet C), both diets having a lower fibre content than diet A of the colobus. Loose faeces is frequently observed in captive colobines, causing weight loss and/or gastrointestinal discomfort (NIJBOER et al 1995, NIJBOER et al 2001).

The present trials aimed at evaluation of stool quality and the apparent digestibilities of crude protein, crude fat, dry matter, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), and non-structural carbohydrates (NSC) in the two different species of colobines.

Materials and methods

Animals and housing

A digestibility trial was performed with 5 captive-borne, adult black and white colobus monkeys. The group consisted of a 24 years old female (estimated weight (e.w.) 8 kg); a female aged 5 years (e.w. 7 kg) and a female of 1 year (e.w. 2.5 kg). The oldest male was 8 years (e.w. 10 kg) and the youngest was 3 years old (e.w. 5 kg). The colobus monkeys at Rotterdam Zoo were housed in an inside (4.20 x 3.85 x 3.65 m) and outside enclosure (7.90 x 4.70 x 3.65 m). The two enclosures were connected by a corridor, the monkeys had free access to both enclosures. The temperature of the inside enclosure was kept at 20 ^{0}C during the test period (September/October 1999) and the relative humidity was about 80 %. From 07.00 to 19.00 hours, the inside enclosure was artificially lighted. Normally, the enclosure floors consist of concrete covered with a layer of wood shavings. During the test period the wood shavings were absent. The diet pellets and fruits were offered in galvanized pans, and the browse willow (*Salix spp*.) and rose (*Rosa spp*.) were put in a large plastic basket.

The other digestibility trial was performed with three spectacled leaf monkeys. The age of the male (e.w. 10.5 kg) was 12 years. The two females were aged 5 (e.w. 8 kg) and 4 years (e.w. 4 kg). The group was housed in an indoor room (5.15 x 2.45 x 3.60 m). The average temperature was 19.5 ^{0}C and the relative humidity was 80 %. Artificial lighting of the facility was on from 07.00 – 19.00 hours. The enclosure floor was made of concrete.

Diets and sampling of test material

There were three different diets: A, B and C. The colobus monkeys received diet A and the spectacled leaf monkeys were fed diet B followed by diet C. During the feeding of each diet there were two test periods of 7 days each. The periods A_1, B_1 and C_1 were followed by periods A_2, B_2 and C_2 respectively, the interval between the two periods being about one week. The trial schedule can be found in Table 1. During the test periods the amounts of feedstuffs offered and the leftovers were weighed for 7 days.

The colobus monkeys (diet A) were fed 3 times a day (08.00, 11.00 and 15.00 hours). In the early morning they received the pellets. At 11.00 hours they received the greens, fruits and vegetables and in the afternoon they received the browse (whole willow and rose). When diet B was fed, the spectacled leaf monkeys received willow leaves at 8.30 hours and the pellets and other grain products at 10.00 hours. At 14.00 hours the spectacled leaf monkeys received their remaining diet.

Table 1. Trial schedule

Animals	Diet	Test period
Colobus monkeys	A	A_1: 20-27 Sept 1999
		A_2: 7-14 Oct 1999
Spectacled leaf monkeys	B	B_1: 4-11 Oct 1999
		B_2: 18-25 Oct 1999
	C	C_1: 10-17 March 2000
		C_2: 22-28 April 2000

During the feeding of diet C, the spectacled leaf monkeys received the pellets at 08.30 hours. At 10.00 hours they received defrosted rose leaves and at 14.00 hours the remaining diet. During period C_2 the pellets were mixed with other grain products like St. John's bread (*Ceratonia siliquia*) in the food bowl in order to improve the palatability.

Table 2 represents a summary of the ingredient composition of the diets offered. To the colobus monkeys (diet A) fennel, celery and endive were offered as greens. The spectacled leaf monkeys received a mixture of celery, cucumber, endive, leek and cabbage as greens when fed diet B, and endive, chicory, fennel, celery, cucumber and lettuce when fed diet C. The colobus (diet A) received apples, oranges and bananas as fruits. The spectacled leaf monkeys were given apples, bananas, grapes, kiwi and melon with diet B and apple, banana, kiwi, grapes and oranges with diet C. As vegetables in diet A, carrots, green pepper and beetroot were used. In diet B, the vegetables consisted of carrot, bean sprouts, broccoli, tomato, green pepper and with diet C French beans, carrot, green pepper, beetroot, broccoli and bean sprouts were supplied.

Table 2. Ingredient composition of the diets A, B and C as determined during the test periods and expressed in g ingredient/kg of whole fed diet. Diet A was fed to the colobus monkeys and diets B and C to the spectacled leaf monkeys.

Period:	A_1	A_2	B_1	B_2	C_1	C_2
Pellets[1]	99	96	48	62	78	71
St. John's bread	-	-	30	14	-	28
Rice	-	-	-	-	69	6
Bread	-	-	-	25	1	-
Leaf-eater vitamins[2]	0.4	0.4	-	-	-	-
Muesli[3]	-	-	24	29		9
Grain flour[4]	-	-	20	18	-	16
Rose leaves	165	218	-	-	66	69
Willow leaves	190	226	65	64	-	
Greens	170	133	212	163	424	324
Fruits	208	198	388	352	104	107
Vegetables	160	122	146	199	230	321
Tofu	-	-	-	-	28	49
Chicken egg, boiled	8	7	16	14	-	-
Meat	-	-	41	60	-	-
Mealworms	-	-	10	-	-	-

[1]The pellets in diet A consisted of Mazuri old world monkey pellets. (Witham, UK; crude protein, 16.3 %; crude fat, 6.1 %; crude fibre, 4.4 %; sugars, 7.9%; starch, 40.9%) and langur pellets (Hope Farms, Woerden, The Netherlands; crude protein, 18.1 %; crude fat, 5.8 %; nitrogen-free extract, 11.1%; NDF, 48,2 %; ADF, 26.4 %; acid detergent lignin, 12.6%) in a 1:1.25 w:w ratio. To both pellets vitamins and minerals were added.
The pellets in diet B were breeding and maintenance pellets for primates (Hope Farms B.V. Woerden, The Netherlands; crude protein, 18.5 %; crude fat, 5.6%; crude fibre, 3.1%, vitamins and minerals added). For diet C the langur pellets (Hope Farms, Woerden, the Netherlands) were offered.
[2]Leaf-eater vitamins: vitamin-mineral supplement developed for colobines (38.7 % Mervit sporavit 325, 60.5 % calcium monophosphate, 0.8 %, vitamin E 500: Pre Mervo, Utrecht, The Netherlands)
[3]Dokkumer muesli, Hedo B.V. Dokkum. The Netherlands. (crude protein, 11.4 %; crude fat, 3.0 % crude fibre, 2.5 %)
[4]Bambix, Nutricon N.V., Zoetermeer, The Netherlands. (crude protein, 11.4 %; crude fat, 1.5 %; crude fibre, 10.5%)

In order to determine the apparent digestibility of the macronutrients, the feed offered as well as the leftovers were weighed. The faeces were gathered and weighed daily during each test period. To analyze the provided diet, the double-meal technique was used. After weighing all ingredients, one meal was given to the animals and the other, identical meal was frozen. The leftovers and the faeces were also frozen until analysis.

Stool quality was scored during the test period on a scale of 1-5. The definitions of the scores are: 1 = properly shaped and generally solid, 2 = properly shaped, 3 = in part properly formed, 4 = loose faeces, and 5 = liquid faeces (NIJBOER 2001). The properly shaped faeces of François langurs, as mentioned in the paper of Nadler (1994) were used as reference.

Chemical analysis

All samples were dried at 60^0C and pooled per period and then ground through a 1 mm screen. Samples of the provided diets, diet leftovers and faeces were analyzed for dry matter, crude protein (nitrogen mass x 6.25), crude fat, ash and crude fibre according to the Weende analyses. The nitrogen free extract (Nfe) is defined as dry matter minus ash, crude protein, crude fat and crude fibre and may contain starch, enzymatically digestible sugars as well as non-digestible carbohydrates. Part of the hemicellulose can be found in this fraction.

NDF, ADF and acid detergent lignin (ADL) were analyzed using the sulphuric acid method described by Van Soest (1982). Hemicellulose was calculated as the difference between NDF and ADF and cellulose as the difference between ADF and ADL. The fraction of NSC is defined as the dry matter minus ash, crude protein, crude fat and NDF; this fraction does not contain hemicellulose.

Results

The amount of nutrients supplied and consumed are presented in Table 3 per animal per day for each of the test periods. The colobus monkeys (diet A) consumed 71% of the dry matter offered. Faeces production was 795 g/day for the whole group (n=5) with a dry matter percentage of 37 %. Of the total faeces, 10% was considered as properly formed, but soft, and 90 % was classified as properly formed and generally solid. The colour of the faeces was dark brown.

From Table 3 it can be calculated that the spectacled leaf monkeys consumed 95 % of the offered dry matter during the two B test periods. Faeces production during the B period was 108 g/day for the 3 animals. The dry matter percentage of the faeces was 21 %. Roughly 10 % of the faeces were considered as dry, 70 % as soft and shapeless and 20 % resembled watery diarrhoea. The colour of the faeces varied from bright yellow to dark green.

Table 3. Amounts of nutrients supplied and consumed by the black and white colobus monkeys and spectacled leaf monkeys during the different test periods. Data are expressed as g/animal/day. Diet A was fed to the colobus monkeys and diets B and C to the spectacled leaf monkeys.

	Supplied		Consumed		Supplied		Consumed		Supplied		Consumed	
Period:	A_1	A_2	A_1	A_2	B_1	B_2	B_1	B_2	C_1	C_2	C_1	C_2
Dry matter	232	250	160	183	138	131	127	129	115	147	89	133
Water[1]	701	684	500	536	558	381	553	380	585	658	559	647
Crude ash	18	18	13	15	7	6	6	6	9	9	7	8
Crude protein	39	39	33	34	27	29	25	28	25	28	18	26
Crude fat	9	9	7	8	7	6	7	6	7	8	6	8
Crude fibre	64	75	34	40	13	7	12	7	22	24	17	22
Nfe	102	109	73	87	84	83	77	82	53	78	41	69
NDF	111	127	70	78	37	36	33	35	34	43	25	39
ADF	90	95	54	55	20	14	17	13	26	33	20	30
ADL	32	30	19	11	13	7	11	7	5	11	4	10
Hemicellulose	21	32	16	23	17	22	16	22	8	10	5	9
Cellulose	58	65	34	36	7	7	6	6	21	22	16	20
NSC	55	57	37	48	47	47	56	54	40	59	33	52

[1] Not including drinking water

When diet C was fed to the spectacled leaf monkeys 85 % of the dry matter supplied was consumed. The dry matter intake during period C_2 was comparable to that during both B periods, whereas during period C_1 it was lower.

The dry matter amount of faeces during period C, was 73.5 g/day, which is less than the output during test period B. The dry matter percentage of the faeces on diet C was 26 %. On diet C, 20 % of the faeces were considered as dry and 80 % as soft and shapeless. No watery diarrhoea was seen. The faeces colour was mostly brown and sometimes green. The stool quality after feeding diets A, B and C is illustrated in Figure 1.

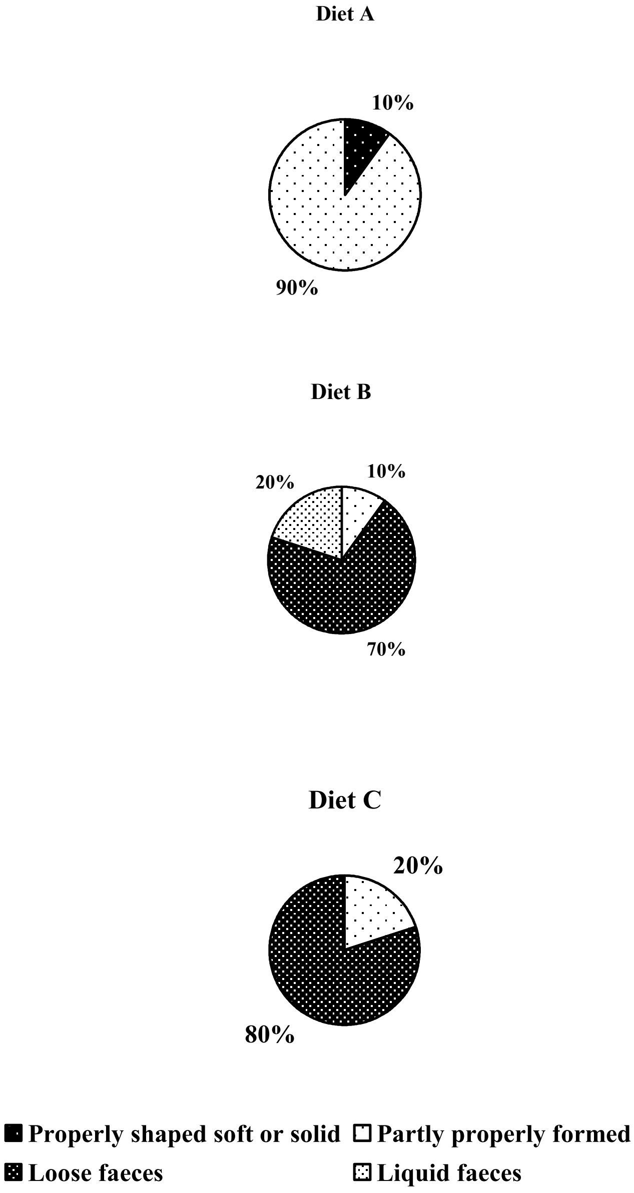

Figure. 1. Proportional distribution of stool quality after feeding the diets A, B and C.

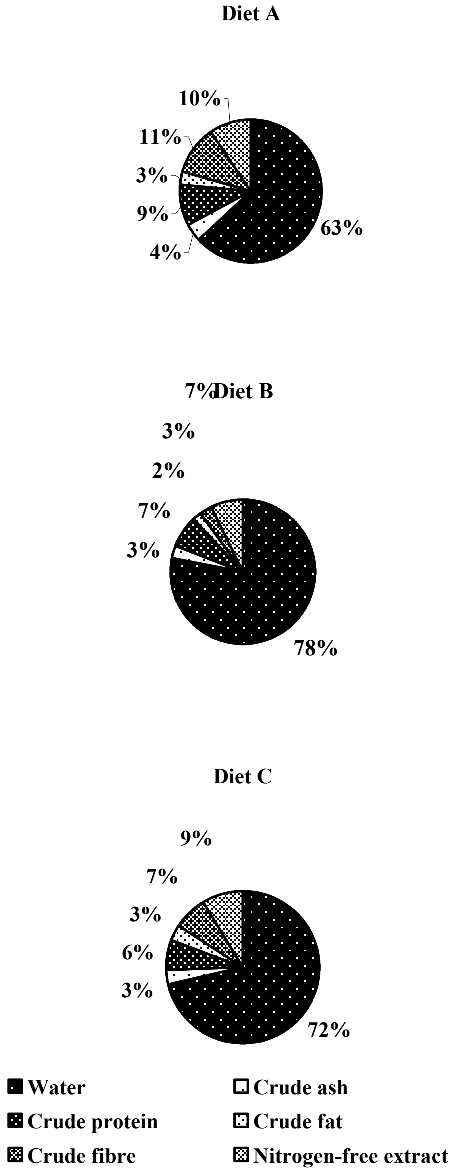

Figure 2. Proportional composition of the faeces

Figure 2 shows the chemical composition of the faeces. The percentage of water in the faeces for diet A is lower than for diets B and C. The percentages of crude protein, crude fibre and carbohydrates in the faeces of the colobus monkeys fed diet A were higher than those in the faeces of the spectacled leaf monkeys fed either diet B or C, whereas the percentages of crude ash and crude fat were comparable for all three diets. The percentage of crude fibre in the faeces for diet C was more than two times higher than that for diet B. The percentage of carbohydrates in faeces for diet C was also higher than that for diet B.

Table 4. Composition of the diets consumed by the colobus monkeys (diet A) and the spectacled leaf monkeys (diets B and C) during the different test periods. Data are expressed as percentage in the dry matter.

Period:	A_1	A_2	B_1	B_2	C_1	C_2
Crude ash	8.0	8.0	4.7	4.7	7.9	6.0
Crude protein	20.4	18.4	19.7	21.7	20.2	19.5
Crude fat	4.6	4.5	5.5	4.7	6.7	6.0
Nfe	45.7	47.5	63.8	63.6	46.1	50.3
Crude fibre	21.3	21.6	9.4	5.4	19.1	16.5
NDF	43.8	42.7	26.0	27.1	28.1	29.3
ADF	33.6	30.0	13.4	10.1	22.5	22.6
ADL	12.1	10.5	8.9	5.4	4.5	7.5
Hemicellulose	10.2	11.8	12.6	17.1	5.6	6.8
Cellulose	21.5	19.5	4.7	4.7	18.0	15.0
NSC	23.2	26.4	34.6	36.4	37.1	39.1

The protein content of the diets consumed varied between 18.4 and 21.7 % in the dry matter, and the fat content between 4.5 and 6.7 %. (Table IV). Diet A contained higher levels of crude fibre, NDF, ADF, ADL and cellulose than diets B and C. The percentage of Nfe in diet A of the colobus monkeys was comparable to that in diet C of the spectacled leaf monkeys, but the percentage of Nfe in diet B was much higher. The content of NSC in diet A was much lower than that in diets B and C. Diet C contained higher levels of ADF than diet B.

The apparent digestibilities of dry matter, crude protein and crude fat were higher for the spectacled leaf monkeys fed either diet B or C than for the colobus monkeys fed diet A (Table 5). Apparent digestion of Nfe was similar for all three diet tests and so was the digestion of NSC, which was nearly complete. The apparent hemicellulose digestion was higher for diet B than for diet C. The apparent digestion of cellulose was lower for the colobines fed diet A than for the spectacled leaf monkeys fed diet C. The data in Table V would indicate that a significant amount of lignin (ADL) is digested on each diet.

Table 5. Apparent digestibility of macronutrients for diets A, B and C as determined during the test periods. The digestibilities are expressed as % of the intake. Diet A was fed to the colobus monkeys and diets B and C to the spectacled leaf monkeys.

Period:	A_1	A_2	B_1	B_2	C_1	C_2
Dry matter	65	68	91	84	78	78
Crude protein	57	55	68	71	88	73
Crude fat	41	41	61	67	50	75
Crude fibre	52	54	75	57	65	68
NDF	56	63	70	77	74	64
ADF	56	56	53	54	65	60
ADL	47	45	55	43	25	40
Hemicellulose	56	79	88	86	60	78
Cellulose	62	61	50	83	75	70
Nfe	80	82	88	93	88	85
NSC	98	91	98	100	97	94

Discussion

In an earlier report (NIJBOER et al 2001) on the effect of diet on the consistency of faeces produced by captive François langurs we have noted the various limitations in studies like the present one. This study is also difficult to interpret because we used two different species of colobines, which were fed on different diets. All data collected during each period is the average for 7 days. It is not realistic to perform statistical analyses. Nevertheless, we feel that the present data are relevant in view of their unique nature, their value in comparing and contrasting them with the scarce literature data and the application in the practical feeding of colobines in zoos.

We are aware of only two studies of the digestibility of macronutrients by black and white colobus monkeys. Oftedal et al (1982) and Watkins et al (1985) have fed diets containing about 10 % ADF and the apparent digestibility of the ADF fraction was found to be about 69 %. This digestibility value is higher than that found in this study. The amount of ADF in this study may not be very important as to ADF digestibility. Diets A, B and C contained 32, 12 and 23 % ADF, respectively, but the ADF digestibility was similar for the three diets with a tendency to be higher for diet C with the intermediate ADF level. The digestibility of the Nfe fraction as observed by Oftedal et al (1982) and Watkins et al (1985) was similar to that found in this study.

As far as we know there are no literature data on macronutrient digestion by spectacled leaf monkeys. Sakaguchi et al (1991) fed silver langurs a diet containing 38 % NDF and 15 % ADF and reported that NDF and ADF digestibility were 69 and 62 %, respectively. These digestibility values correspond with those seen for diet C.

In keeping with our study on François langurs (NIJBOER et al 2001), the black and white colobus and the spectacled langurs displayed apparent digestion of lignin. Similarly, Oftedal et al (1982) and Watkins et al (1985) also found lignin digestion in colobus monkeys. Possibly colobines have intestinal bacteria or fungi capable of breaking down lignin.

It is clear that colobines efficiently utilize dietary fibre. However, it is not known to what extent fermentation takes place in the fore stomach through cellulolytic bacteria breaking down cellulose and hemicellulose molecules. Such a breakdown would serve a vital function in liberating the readily digestible contents of plants. Fermentation would also yield volatile fatty acids, which not only provides energy, but also propionic acid, a precursor of glucose synthesis. Wild shot colobus monkeys had a relatively low fore stomach pH and showed no evidence of cellulolysis, but they had eaten fruits and seeds, which can be fermented rapidly causing a reduction of pH and cellulolytic activity. (KAY & DAVIES 1994). This condition would impair the utilization of dietary fibre. Free ranging leaf-eating monkeys spend large parts of the day foraging. This strategy of dispersing feeding bouts over time reduces the sudden influx of rapidly fermentable material. Therefore it is suggested to feed captive animals at least 4 times a day. The gradual distribution of smaller amounts of feed during the day improves a steady fermentation and helps to ensure adequate dispersal of feed among individuals housed in a group (EDWARDS & ULLREY 1997).

Apparent crude protein digestibility ranged from 55 to 88 %. For black and white colobus monkeys values of 83 % (OFTEDAL et al1982) and 78 % (WATKINS et al 1985) have been reported, and for silver langurs a protein digestibility of 83 % was published (SAKAUCHI et al1991). Protein digestibility is determined by protein digestibility and accessibility for digestive enzymes. Crude fat digestibility ranged from 41 to 75 %, which is lower than the value of 84 % reported by Watkins et al (1985). The low apparent fat digestibility could relate to the low dietary fat concentrations so that endogenous faecal fat may have had a depressing influence.

The spectacled leaf monkeys were fed two different diets. When changing from diet B to diet C, rose leaves replaced the willow leaves, because willow leaves were no longer available. The willow leaves contained 63% water and had 34 % NDF, 26 % ADF and 14 % lignin in the dry matter fraction. The rose leaves contained 73% water and had 31 % NDF, 18 % ADF and 4 % lignin in the dry matter. Because the rose leaves contain less fibre than willow leaves, French beans were added to diet C. An increase in the amount of fibre in diet C was also achieved by the addition of langur pellets. The result was that with diet C the NDF content increased from 27 % to 29 % and the content of ADF increased from 12 to 23 %. As a result, the amount of hemicellulose declined from 15 % in diet B to 6% in diet C and the cellulose percentage raised from 5 to 17 %. The amount of NSC was similar in diets B and C. The increase in fibre intake with diet C versus diet B was associated with a decrease in the apparent digestibility.

Faeces consistency of the black and white colobus monkeys fed with diet A was better than for the spectacled leaf monkeys, irrespective of the diet that was fed. The formation of soft and shapeless faeces, including watery diarrhoea was directly related with the water content of the faeces. When the spectacled leaf monkeys were switched from diet B to diet C, the intake of fibre increased which was associated with an improvement of the faeces quality. A similar observation was made in our previous study with François langurs (NIJBOER et al 2001). In the present study the improvement of faeces quality in spectacled leaf monkeys fed diet C instead of diet B showed also an increase in the Nfe content. We have speculated earlier (NIJBOER et al, 2001) that the extra fibre ingested may have been partly fermented, leading to the recovery of the breakdown products in the Nfe fraction of the faeces. The breakdown products could have a superior water-binding capacity, resulting in the production of well-shaped faeces. Alternatively or additionally, the increase in the amount of fibre in faeces may be responsible for the improvement of the faeces quality. The colobus monkeys fed with diet A had the highest amount of fibre in their faeces and also had superior faeces quality. For all three diets this study clearly shows that high intakes of crude fibre are correlated with high amounts of crude fibre plus Nfe in faeces. In agreement with our earlier suggestion (NIJBOER et al 2001) we suppose that the soft faeces and diarrhoea frequently seen in captive colobines could relate to a low intake of fibre. Crude fibre contents in the dietary dry matter as high as 25 % may be necessary for the production of well-shaped faeces. It is reassuring that colobines have been shown to digest dietary fibre efficiently.

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Zusammenfassung

Verdauungsversuche wurden durchgeführt an Guerezas (*Colobus guereza*) und Brillenlanguren (*Trachypithecus obscurus*) um Zusammenhang zu finden zwischen Futterzusammensetzung, augenscheinlicher Hauptnährstoffverdauung und Qualität der Fäkalien. Fäkalien produziert von Guerezas wurden betrachtet als gutgebildet und im allgemeinen fest.

Die Brillenlanguren wurden Diäten gefüttert mit verschiedenen Mengen Rohfasern, NDF und ADF. Die Brillenlanguren, welche ernährt wurden mit geringern Mengen Fasern in ihren Diäten als diejenige in der Nahrung der Guerezas produzierten im allgemeinen weiche und unförmliche Fäkalien und ab und zu sogar wässerige Diarrhöe. Eine Zunahme der Menge Rohfaser und Zellulose in der Nahrung der Brillenlanguren ergab sich in eine Besserung der Fäkalienqualität. Was die drei Diäten und die zwei studierten Affenarten anbelangt, wurde klar daß Aufnahmen mit höheren Mengen Fasern und Zellulose in Zusammenhang stehen mit höheren Mengen von Rohfasern plus Kohlenhydraten in den Fäkalien.

Augenscheinliche Verdauung von Rohfasern in den Guerezas und den Brillenlanguren bezifferte sich auf 52 – 68 % der Aufnahmen. Dieses Studium zeigt daß eine hohe Aufname von Rohfasern, Zellulose inbegriffen, bei Brillenlanguren einen bessere Qualität der Stuhlgang abwirft.

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CHAPTER 8

Effect of diet on the feces quality in Javan langurs (*Trachypithecus auratus auratus*)

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Abstract

A high intake of easily fermentable carbohydrates, and a low intake of fiber material, is generally regarded as a major factor affecting the health of captive langurs. The effect of excluding fruits and vegetables from the diet on fecal consistency 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, containing 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 suggest 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 aur*atus *auratus,* Javan langurs, feeding, nutrition, fecal score, fecal consistency, digestive upset, dietary fiber

Introduction

Javan langurs (*Trachypithecus auratus auratus*) belong to the primate sub-family of Colobinae, which are part of the family Cercopithecidae. Colobines are folivorous and have a multi-chambered 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.[2,3]

The natural habitat of the Javan langurs is in Java, and their natural diet consists of young leaves, fruits and flowers[7,9] although recent guidelines recommend the use of high-fiber primate biscuits, green leafy vegetables and browse[5] 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.[13,14] Such a feeding regime may result in gastro-intestinal disturbances[7] and often leads to loose stools.[11] Commercial fruits and vegetables have been cultivated to comply with human taste and therefore are high in soluble sugars[8,10] and low in fiber.[16] 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.[4]

In captive François langurs (*Trachypithecus françoisi*), it was shown that stool quality was improved when the fiber content of the diet was increased.[12] In that study, the intake of extra fiber was associated with lower consumption of non-structural carbohydrates. Thus, it could be suggested that a decrease in the intake of non-structural 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 of fruits and vegetables from the usually fed diet on stool quality.

Materials and methods

Animals 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. There were a male and two female adults, a female subadult and a male juvenile. The animals were kept in an inside enclosure of 63 m^2. They had access to an outside island with a surface of 56 m^2. During the experimental period, the langurs went outside only a few times. Due to the season, there were no palatable plants in the outdoor enclosure. The temperature in the inside enclosure was 18 ^{0}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 07:00 to 19:00 h. The food was offered in a wooden trough (0.8 x 0.5 x 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 through (0.4 x 0.4 m). At the Apenheul Zoo, the group of Javan langurs consisted of four adults (1 male and 3 females), four sub-adults 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^2, the two enclosures being connected by a tunnel. The langurs also had access to outdoor enclosures of 250 and 12 m^2 in which no plants were available for consumption. The temperature in the inside enclosure ranged between 18 and 20 ^{0}C. The humidity was about 80 %. Artificial light was on from 08:30 to 16:30 h. Food was offered in troughs (2.0 x 0.5 x 0.1m).

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_1 from 6-10 January, A_2 from 23-27 January and A_3 from 2-6 February). The traditional diet was fed during the A_1 and A_3 period. During the A_2 period the vegetables were left out during the trial.The traditional diets were fed at Apenheul Zoo during periods B_1 and B_3. During period B2 the vegetables and fruits were left out. Diet B_1 was fed from 5-11 January, B_2 from 20-26 January and B_3 from 10-16 February. The time between the observations was used to adapt the animals.

At the Rotterdam Zoo, the amounts of pellets and browse were ad libitum fed during all periods. In the traditional diet, the amount of vegetables was also not restricted. Pellets and rice balls with vitamins were fed at 8:00 h, the browse at 10:30 h, vegetables at 13:30 h and the remaining amount of browse at 15:45 h during the A_1 and A_3 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_2 3.8, vitamin B_6 0.4, vitamin $B_{12;}$ and in IU/g: vitamin A 961, vitamin D_3 192, 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_2 period, the primates received the pellets and rice balls at 8:00 h and the stripped rose and willow leaves at 11:00 h. During the adaption period from diet A_2 to diet A_3 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, less vegetables were offered in A_3 as compared to A_1 to reduce waste; this did not influence vegetable intake.

During periods B_1 and B_3 at the Apenheul Zoo, pellets (Mazuri Leaf Eater Pellets, Mazuri, Witham, United Kingdom), greens and sunflower seeds were fed at 9:00 h; browse at 12.00 h; beans and vegetables at 14:00 h and the remaining browse, cheese, nuts, yogurt and fruits at 16:00 h. 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 foodstuffs such as eggs, cheese, chicken fillet and yogurt were given. During period B_2 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 analyse 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 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.[21]

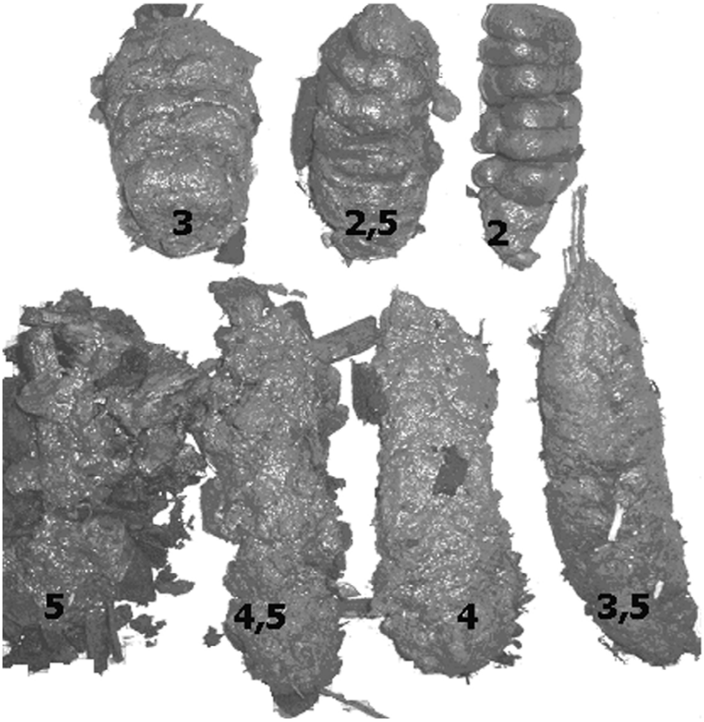

Figure 1: Feces scoring system for langurs

Grade 1 feces are firm and dry whereas grade 5 equals 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.[11] 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 ^{0}C to constant weight, pooled per period and ground to pass though a 1-mm screen. Samples of the diet offered and diet leftovers were analyzed for dry matter, crude protein (nitrogen x 6.25), crude fat, ash, and crude fiber, according to the Weende methods.[1] The nitrogen free extract (Nfe) is defined as dry matter minus ash, crude protein, crude fat and crude fiber. Neutral detergent fiber, ADF and acid detergent lignin (ADL) were analyzed according to the method described by Van Soest.[19,20] Hemicellulose was calculated as the difference between NDF and ADF. Cellulose is the difference between ADF and ADL. The fraction of non-structural carbohydrates (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 (INSTAT 3, GraphPad Sofware Inc., San Diego). Regression analysis and Spearman's Correlation Coefficient (SCC) (SPSS 12.0, Chicago, Illinois) served to evaluate the relationship between 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 diet 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_2 and B_2 the amounts of browse ingested were markedly higher than during periods A_1 and A_3 and periods B_1 and B_3, respectively.

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

Feeds	Diets A						Diets B					
	A_1		A_2		A_3		B_1		B_2		B_3	
	S	I	S	I	S	I	S	I	S	I	S	I
Pellet[1]	196	49	332	183	225	142	57	57	93	93	51	51
Browse[2]	491	177	1036	456	816	294	375	240	592	379	364	233
Vegetables[2]	815	563	0	0	720	576	903	894	0	0	943	895
Fruits[2]	-	-	-	-	-	-	18	18	0	0	29	29
Rice balls[2]	102	101	61	61	87	86	-	-	-	-	-	-
Other[3]	-	-	-	-	-	-	24	24	45	45	36	36

[1] The langur pellet (Hope Farms B.V., Woerden, The Netherlands) fed as component of diets A, contained, on a 90 % dry matter basis, crude protein, 18.1 %, crude fat, 5.8 %, Nfe, 11.1 %, NDF, 48.2 %, ADF, 26.4 %, ADL, 12.6 % and added vitamins and minerals. The pellet (Mazuri Leafeater Pellet, SDS, Witham, UK) 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).
[2] See Methods for details.
[3] Cheese, nuts, yogurt, eggs, chicken fillet

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

	Diets A			Diets B		
	A_1	A_2	A_3	B_1	B_2	B_3
Dry matter intake	154	256	268	207	241	238
Water intake[1]	736	468	830	1026	276	1006
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

[1] Water ingested with food items; does not include drinking water.

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_2 and B_2. Out of the six fiber 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_2 and B_2 were 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_2 and B_2 were fed as compared to the other diets.

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

	Diets A			Diets B		
	A_1	A_2	A_3	B_1	B_2	B_3
Number of fecal boli	102	140	100	364	374	376
Mean score[1]	3.42[a]	2.57[b]	3.33[a]	3.60[a]	2.69[b]	3.19[c]
S.D.	0.75	0.64	0.75	0.43	0.78	0.50
Most common score	4	2	4	4	2.5	3.5

[1] Means within a facility with different superscripts differ significantly at p<0.001 (Kruskal-Wallis and Dunn's post hoc test).

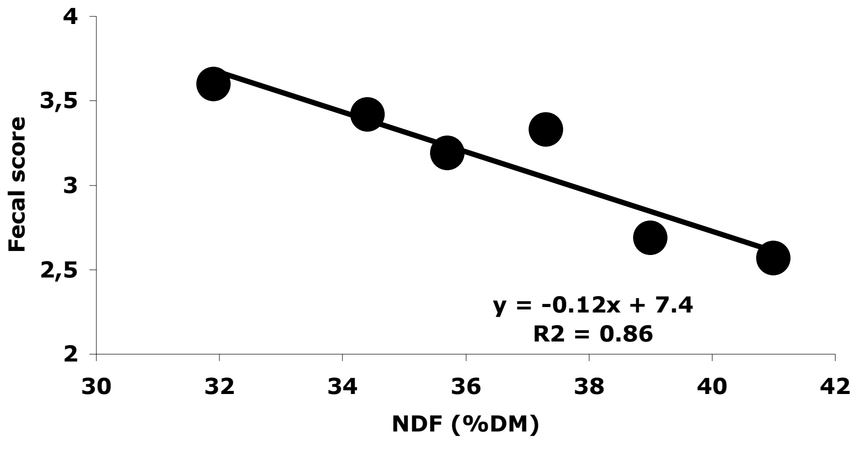

Figure 2. Correlation between the mean fecal score and the dietary concentrations of NDF.

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 observed in the mean fecal score. The regression equation is: score = 5.05 – 0.08*NDF + 0.01*(water content).

The non-significance of the coefficients (NDF: p=0.118, water content: p=0.277) is due to the collinearity 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 due to the close sequence of the trial periods, the approach of using average intake values is justified.

In an earlier study with François langurs,[12] 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 pattern in the langurs' forestomachs. Due to its high sugar content, produce, and especially fruits, can lead to a very rapid or even "explosive" fermentation.[15] 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.[17] 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 % DM would be necessary to guarantee optimal fecal consistency. This value lies within the range of NDF content provided [13] for both NDF in native colobine foods (ranging from 44 to 67 %DM) and 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_2 and B_2 were fed. The decrease in intake of soluble sugars after feeding diets A_2 and B_2 was not illustrated by chemical analysis of the diets, most likely demonstrating an analytical shortcoming of the methods employed. Whereas it can be expected that the NSC fraction of commercial fruits and vegetables contain mainly sugars, the NSC fraction of browse is more likely to consist of other carbohydrates such as pectins[6]. Therefore, a change of NDF-soluble carbohydrate sources that would be beneficial for the fiber-digesting symbiontic forestomach 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$^{0.95}$.[18] 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. On the conventional zoo diets, average daily water intake (740-1030 ml) surpassed this range considerably. Although one would expect that excess water 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 suggest 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[5] with 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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CHAPTER 9

Influence of two different diets on fluid and particle retention time in Javan langur (*Trachypithecus auratus auratus*)

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Zoo Biology

Abstract

In captivity, langurs (foregut fermenting primates) often suffer from digestive disorders, and in particular display soft stools on diets with a high proportion of fruits, vegetables, and grain products. In this study, we tested whether the improvement in feces consistency expected after an omission of vegetables from a conventional diet was also accompanied by a change in the excretion pattern as measured by the mean retention time (MRT). Two adult Javan langurs were kept together and offered a conventional diet of pellets, browse, vegetables, or a diet consisting of pellets and browse only. MRT were measured with cobalt-EDTA as the fluid and chromium oxide as the particle marker before, during, and after the test diet, collecting the feces of both animals together. MRTs of fluids and particles were longer (47/49 h) on the test diet as compared to the usual diet (42/43 h). Feces consistency improved during the test period. The test period was marked by an increased fiber intake; however, on the conventional diet, dietary water intake exceeded the calculated water flux for these animals distinctively, due to the high proportion of vegetables. Therefore, it cannot be decided whether the increase of fiber level alone or also a reduction of the excessive water intake was responsible for the changes observed. Comparing the results of this study to other studies on ingesta retention in foregut fermenting primates, no correlation between the body mass of the animals and the MRTs measured is evident, which is in accordance with observations in other groups of foregut fermenters.

Introduction

Historically, langurs have been very difficult to keep successfully over an appropriate individual time span. Edwards (1995) summarized anecdotal and studbook data that showed that captive langurs had a dramatically short lifespan in captivity as compared to other primates, and as compared to what would be expected to be an average lifespan in the wild. Most of the problems associated with langur husbandry have been related to diet (Hölihn, 1973; Merrit, 1983; Edwards, 1990; Nijboer et al., 2006a).

Langurs are peculiar among the primates as they are foregut fermenters (Bauchop and Martucci, 1968; Kuhn, 1964; Milton, 1998). Traditionally, herbivorous or frugivorous primates were fed diets in captivity that are drastically higher in soluble carbohydrates and lower in fiber components than their natural forage (Edwards, 1990; Nijboer and Dierenfeld, 1996). A major reason for this phenomenon is the misconception that “frugivores” should be fed those fruits easily available to zoos. However, “wild” fruits and commercially available fruits differ enormously in their respective content of soluble carbohydrates and fiber (Conklin-Brittain and Wrangham, 2000; Lawrence et al., 2005). It has been stated repeatedly that, if the nutrient composition of wild fruits is to be mimicked, leafy green vegetables rather than commercial fruits must be fed (Edwards, 1995).

For folivorous primates, especially for langurs, feeding recommendations have been made based on the use of a high-fiber primate pellet, substantial amounts of browse, and green leafy vegetables (Edwards et al., 1997; Nijboer and Dierenfeld, 1996). However, the feeding regimes in many zoological institutions where langurs are kept are not in accordance with these recommendations but still include significant proportions of fruits, colored vegetables, and cereal or milk products in their diets (Nijboer and Dierenfeld, 1996; Nijboer et al., 2006b).

If such diets are fed to monogastric animals where any fermentation that might occur takes place in the hindgut, this will mean that the majority of these easily digestible components (soluble carbohydrates) are digested and absorbed in the small intestine before they can reach the sites of fermentation. The effect of such a feeding practice will be a constant oversupply of energy, a fact reflected in findings in lemurs, which are often exceptionally obese when compared to free-ranging species (Schwitzer and Kaumanns, 2001). In foregut fermenters such as langurs, however, the supply with easily digestible carbohydrates will have a more deleterious effect: similar to rumen acidosis in cattle, this over supplementation will lead to severe disturbances in the foregut microflora and fermentation (Oftedal et al., 1996; Slyter, 1876; Radostis et al., 1999). One of the possible results of a chronic disturbance of the foregut is permanent “loose stools”, a regular observation in many langur facilities (Nijboer et al, 2006a,b).

In two earlier studies, it has been demonstrated that an exclusion of feeds rich in soluble carbohydrates (fruits, colored vegetables, cereal products) and an increase in the use of high-fiber pellets and browse, led to a significant improvement in feces consistency in captive langurs (Nijboer et al., 2006b). A significant correlation between the dietary fiber content and feces consistency could be demonstrated (Nijboer et al., 2006a). However, there was also a significant correlation between dietary water intake and feces consistency. It is most likely that the dietary water intake on convential fruit/vegetable diets exceeds the daily water requirement of the animals. Thus, a postulated effect of the diet change was a different ingesta retention that allowed for more water re-absorption in the gut. Therefore, we performed a simple passage experiment with two Javan langurs on the respective different diets, to test whether differences in fluid and particle retention could be measured.

Methods

Animals and housing

Two adult silver leaf monkeys (estimated body mass 5 and 7 kg) were housed in an inside enclosure which measured 3 m by 7 m. The floor of the enclosure was normally covered with coconut chips. During the test the chips were partly removed in order to collect all feces and leftovers. The enclosure was accommodated with fake trees and branches. Part of the enclosure was provided with natural daylight. The silver leaf monkeys had also access to another inside enclosure (2 m x 3 m) with a concrete floor. This enclosure was completely illuminated by artificial light. In both enclosures the light was automatically turned on at 7.00 am and turned out at 7.00 pm. The temperature in both enclosure was constantly 18^{0}C. Drinking water was available at all times from automatic water pans.

Foods, feeding and sampling

Two different diets (A and B) with an interval of two weeks were tested. Diet A_2 was similar to diet A_1 and was fed two weeks after diet B. The food offered and consumed during test periods A_2 and B in 2004 can be found in Table 1. In each period 150 grams browse a day was offered consisting of different species of leafs and twigs; the additional amount of browse consisted only of leaves of willow (*Salix spp*.) and rose (*Rosa spp*.). The vegetables offered were 100 g endive, 50 g cucumber, 100 g green pepper, 100 g carrots, 50 g beet, 50 g celery, 200 g French beans and 100 g fennel. The composition of the langur pellets can be found in Table 1. Rice balls were prepared from cooked white rice (250 g) mixed with 5 g low–fat yogurt, 5 g fruit syrup and 1 g of vitamin/mineral mixture. During the experimental periods two rice balls (50 g) were fed at 7.30 am, the browse partly at 11.30 and the remaining browse and produce at 4.30 pm. However, during the B period the vegetables were left out.

Table 1: Offered (O) and consumed (C) diet items (g/animal/day) during period A_1, B and A_2 .

Food type	Diet A_1		Diet B		Diet A_2	
	O	C	O	C	O	C
Langur pellets[1]	200	71	350	103	200	65
Browse	420	249	1129	583	493	203
Vegetables	756	621	-	-	751	641
Rice balls	50	46	50	46	53	52

[1] A breeding and maintenance pellets for langurs (Hope Farms B.V. Woerden, The Netherlands; crude protein, 19.8%; crude fat, 6.3%; crude fiber, 28.0.0%).

At the beginning of the A_1, B and A_2 test periods 5,04 ml of a cobalt EDTA solution with suspended chromium (III) oxide particles (85,48 g Co-EDTA and 14,43 g CrO_2/l) were mixed with two rice balls of 50 g. Both animals received a rice ball - which was immediately eaten - at 7.30. After that time all feces in the enclosures were scored as described in Figure 1 and then collected for analysis. On day 1 feces was scored at 11.30, 16.30 and 21.30, on day 2 at 7.30, 11.30, 16.30 and 21.30, on days 3, 4 and 5 scoring took place at 7.30, 11.30 and 16.30 and on day 6 at 7.30 and 16.30. The final scoring took place on day 7 at 7.30 in the morning. All food items offered and left over as well as the total fecal output were weighed. Results were divided by two to give the values per animal.

Chemical analyses

Analyses from period B and A_2 were done from the food offered, the leftovers and the feces at the laboratory of the Department of Nutrition at the Faculty of Veterinary Medicine in Utrecht.

All samples were dried at 60 ^{0}C and pooled per period and then ground through a 1 mm screen. All samples were analyzed for dry matter, crude fiber, crude protein, crude fat and ash using standard laboratory procedures. Apparent digestibility (aD) coefficients were calculated as (Intake – Excretion) / Intake * 100 for dry matter or any given nutrient.

Chromium and Cobalt were analysed by using the method described by Perkin-Elmer (AAS, 1982): drying of samples for 16 h at 105 ^{0}C, dry ashing by combustion for 16 h at 540 ^{0}C, dissolving the remaining ash in 4 M HCL. The resulting solution is then measured by Atomic Absorption Spectrophometry (AAS, 1982). The concentrations of Chromium and Cobalt were used to estimate the transit time (time of the first appearance of the markers in the feces). The mean retention time (MRT) was calculated according to Thielemans et al. (1978).

Feces scoring system

During all periods the feces was evaluated by using a system designed by Waltham Centre for Pet Nutrition (Waltham, 2000). This system has a score from 1 (firm and dry) to 5 (diarrhea). This system was adjusted for langurs; a score of 2 was considered as optimal (Figure 1).

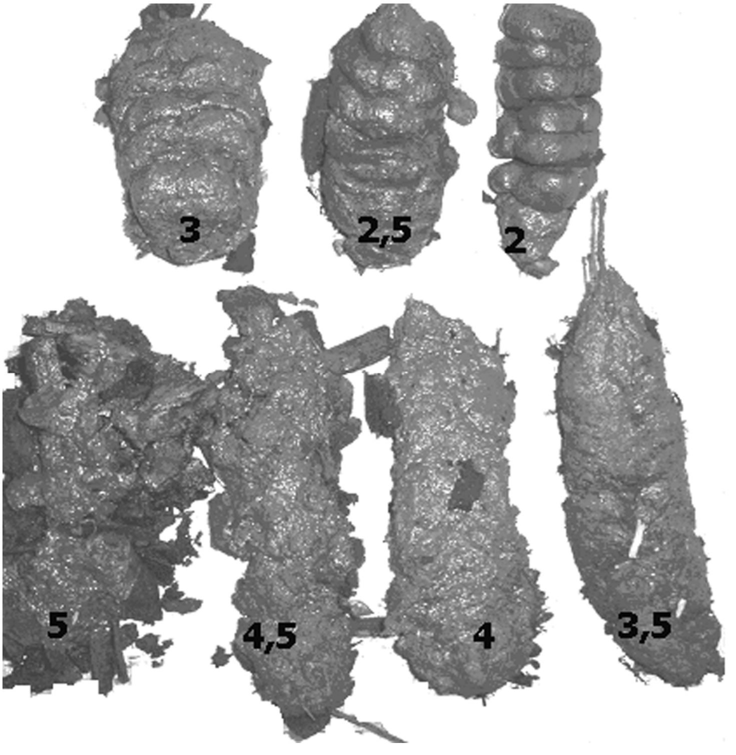

Fig 1. Faces scoring system for langurs.

Results

Dry matter and dietary water intake, the nutrient composition of the ingested diets, and aD coefficients for period B and A_2 are presented in Table 2. Table 2 shows that the amount of dietary water consumed in period B was lower than the amount of water supplied and consumed in period A_2. In period B the intake of crude fiber was higher than in period A.

Average feces production during 6 days of 2 animals was 502 g/day in period B and 494 g/day in period A_2 with an average dry matter percentage of 29.6 % and 23.5 %, respectively. Figure 2 shows the detailed frequency of fecal scores in the three periods. The mean (± S.D.) fecal score was 3.14 (± 0.35; n = 184) for period A_1, 2.78 (± 0.23; n = 240) for period B, and 3.42 (± 0.26; n = 187) for period A_2.

Table 2 : Dry matter intake (DMI) and dietary water intake per animal, ration dry matter (DM) content and nutrient content (%, on a DM basis) of the consumed diet (C) and feces (F), the respective apparent digestion (aD) coefficients of diet B and A_2.

	------ Diet B -----			----- Diet A_2 ------		
	C	F	aD	C	F	aD
DMI (g)	177	72		184	58	
Water[1] (g)	516	179		817	189	
DM (% as fed)	25.5	28.6	59	18.4	23.5	68
Crude protein	13.6	18.5	43	15.7	19.0	62
Crude fiber	28.6	29.4	58	20.8	30.5	54
Crude fat	5.4	6.9	48	2.9	7.1	24
Ash	4.7	10.6	48	6.9	10.7	51
Nitrogen free extract	44.7	27.9	75	48.5	27.4	82

[1] Not including drinking water

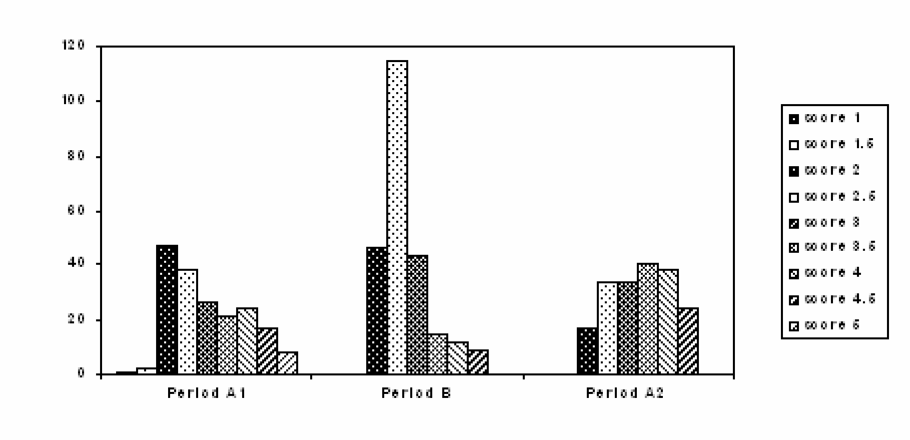

Fig 2. Feces scores during the different periods

Figure 3 shows a graph of marker excretion. The calculated MRTs were, for fluids/particles, 42/43 h in period period A_1, 47/49 h in period B, and 42/43 h in period A_2. In periods A, the first marker excretion was measured in the fecal samples 9 and 14 h after marker ingestion, whereas in period B, the first marker excretion was measured after 24 h.

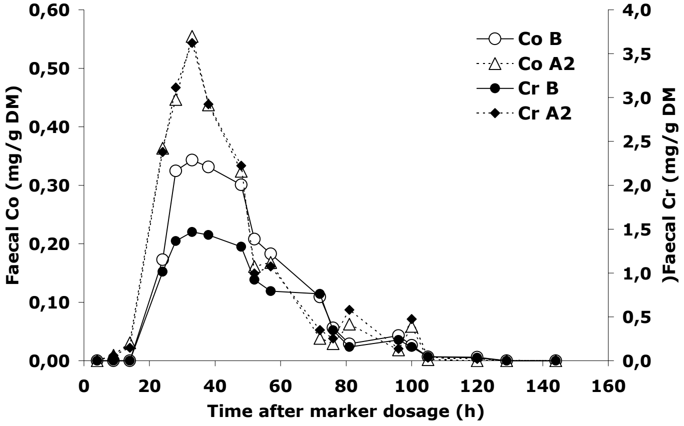

Fig 3. Excretion patterns for fluids and particles at period A_2 and B

Discussion

The finding that the exclusion of vegetables, and an increase of browse and pelleted feeds leads to a better fecal consistency in captive langurs (Nijboer et al., 2006a), was confirmed in this study. These observations underline the importance of the Nutrition Advisory Group feeding recommendation for langurs, which state that fruits, vegetables (except the green, leafy varieties) and energy dense items such as grains or bread should not be used as dietary items for folivorous primates (Edwards et al., 1997).

Apparent digestibility (aD) coefficients for dry matter and crude fiber measured in this study are generally lower than those previously reported. Distinctively higher dry matter, and in particular fiber aD coefficients were mainly reported on diets with even lower fiber content and/or little or no browse supplementation (Oftedal et al., 1982; Robinson et al., 1982; Dierenfeld and Koontz., 1992); in some of these studies, the dry matter intake was – correspondingly – much lower than in the Javan langurs of this study (Oftedal et al., 1982). However, even in a trial where a high-fiber (30 % acid detergent fiber) primate biscuit was the sole ration ingredient, aD for fiber was high (aD ADF 73 % compared to diet B with 29 % crude fiber and aD crude fiber of 58 %) (Edwards and Ullrey, 1999). This is, most likely, also due to the use of browse supplements as a major fiber source in period B, which can be expected to be less available to bacterial fermentation than the finely milled components of manufactured diets. Within our own study, the variation in aD dry matter ($A_2 > B$) is best explained by variation in dietary crude fiber content ($A_2 > B$), as fiber levels are known to repress digestibility in many animal species.

Caton (1999) explained problems encountered in ingesta retention studies in colobines in detail. In contrast to this and similar studies, in our case it was possible to alter the traditionally fed zoo diet to compare two dietary regimes; however it was not possible to measure ingesta retention in individual animals. Usually chromium-mordanted fiber is used as a particle marker alongside with the fluid marker cobalt-EDTA. Cr-mordanted fibers are probably more representative for small plant particles, whereas chromium oxide is dosed as powder particles of a higher density than the usual ingesta. However, in other studies on passage characteristics of foregut-fermenting primates no differences between fluid and particle retention were found that were particularly different from our results. These results suggest that, in contrast to ruminants, selective particle retention does not play a major role in the digestive physiology of langurs.

In ruminants variation of dry matter intake is an important factor influencing ingesta retention, as well as dietary fiber level and the proportion of roughage in the diet (Lechner-Doll et al., 1991). In this study retention of both fluids and particles was distinctively longer on diet B than on diets A, regardless of a comparable dry matter intake. Therefore it could be speculated that dietary fiber content is a decisive factor for MRT in langurs as well – a hypothesis also supported by the data of Edwards and Ullrey (1999b). In their study an individual colobus monkey displayed an increase in particle MRT when the fiber level of the extruded diet was increased, while intake remained constant.

It has been noted earlier that foregut fermenting primates display longer ingesta retention times than expected from interspecific regressions with body mass (Dierenfeld and Koontz, 1992). In particular, they seem to have longer MRTs than hindgut fermenting primates even when consuming higher amounts of dry matter (Edwards and Ullrey, 1999b). These long MRTs are generally interpreted as the cause for the particularly effective dry matter and fiber digestibility in these species (Sakaguchi et al., 1991; Dierenfeld and Koontz, 1992; Caton, 1999; Edwards and Ullrey, 1999b). Such argumentation becomes particularly evident when using primates with much shorter MRTs and lower fiber digestion coefficients as comparison, e. g. lemurs (Edwards and Ullrey, 1999a). When published MRT data for foregut-fermenting primates is plotted against body mass (Fig. 4), it is evident that there is no close correlation between the two parameters. Similarly, no correlation between body mass and MRT was demonstrated for ruminants (Wenninger and Shiley, 2000, Clauss et al. in press), and for the two existing hippopotamus species by Clauss et al. (2004). In combination, these results suggest that, at least among foregut fermenters, body mass is not a reliable predictor of MRT.

In the present experiment, in the period with the longer MRT, fiber intake of the langurs was increased, but water intake was reduced at the same time. According to Robbins (1993), daily water flux in mammals equals 0.159 body $\text{mass}^{0.95}$, which would result in 618 ml/d in a 6 kg-animal such as the langurs of this study. With respect to langurs, one might add that an even lower water flux might be expected due to the generally lower metabolic rate of these animals (Ross, 1992). At more than 800 ml of dietary water per animal and day, diet A_2 – and probably also A_1 - surpassed the calculated water flux distinctively. Water intake from the automatic water pans was not observed during any feeding period during this study. Whether this could be a contributing factor to shorter MRTs and softer feces remains to be demonstrated.

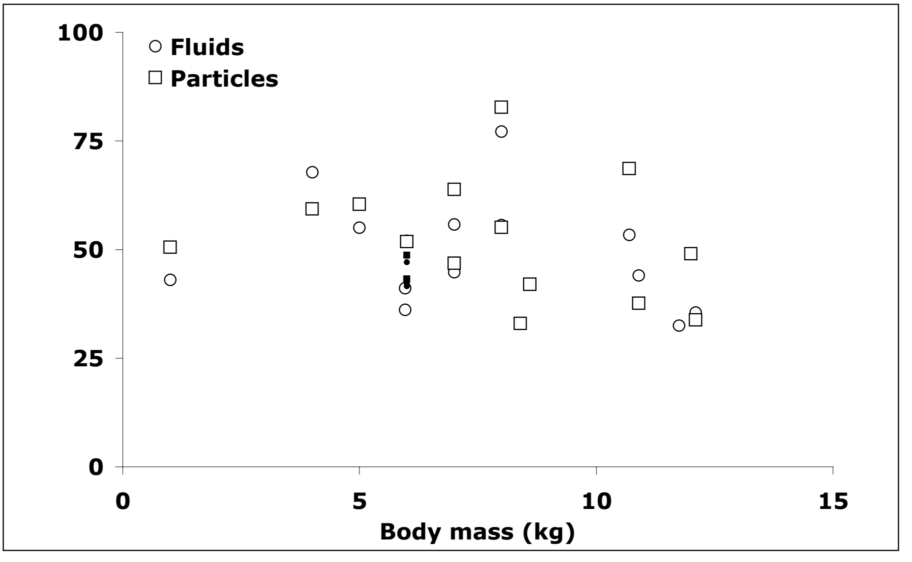

Fig 4. Mean retention time plotted against body mass from foregut-fermenting primates. Data from Dierenfeld and Koontz. (1992), Edwards and Ullrey (1999b), Caton (1999), Kirkpatrick (2001), Sakaguchi (1998). Data of this study indicated by black symbols.

Conclusions

1. A dietary change in two captive langurs, omitting vegetables from their diet, resulted in an increased intake of pellets and browse.
2. Feces consistency improved during the period when vegetables were omitted, and deteriorated again when the usual diet was given again.
3. On the diet without vegetables the mean retention time of both fluids and particles increased as compared to the usual feeding regime.
4. Both an increase in dietary fiber and a decrease in dietary water intake could be responsible for the effects of the diet change.

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CHAPTER 10

Comparison of the fatty acid patterns of blood plasma and erythrocytes in leaf-eating and non-leaf-eating primates fed diets with different fat sources

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Submitted

Abstract

There is evidence that in the stomach of leaf-eating langurs there is microbial fermentation of dietary fibre fractions. This could imply that dietary polyunsaturated fatty acids are hydrogenated in the stomach of langurs as they are in the rumen of ruminants. This idea was put to the test in the present study by comparing the fatty acid composition of plasma and erythrocytes in langurs fed a diet rich in α-linolenic acid as opposed to linoleic acid and by comparing the values in langurs with those in gorillas. After feeding to the langurs the diet rich in α-inolenic acid instead of that rich in linoleic acid, there was a clear increase in the content of α-linolenic acid in both plasma and erythrocytes. The langurs did not have lower contents of α-linolenic acid in erythrocytes than did the gorillas, even though the latter monkeys had higher group mean levels of linoleic acid. These data would point at absence of hydrogenation of polyunsaturated fatty acids in the stomach of langurs. However, especially on the diet rich in α-linolenic acid there was a shift to more stearic and oleic acid isomers in plasma and erythrocytes. This could be interpreted as an indication that in langurs microbial hydrogenation takes place before absorption of the fatty acids.

Introduction

In monogastric species the fatty acid composition of the adipose tissue reflects that of the diet (Beynen et al., 1980; Van Niel and Beynen, 1997; Valero-Garrido et al. 1990). Through the process of hydrolysis and biohydrogenation in the rumen of ruminants, a portion of dietary polyunsaturated fatty acids like linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3 ALA) is converted into saturated fatty acids consisting of steraric acid (C18) and geometrical and positional di- and monoenoic isomers (Noble, 1984). This is also reflected in the fatty acid composition of adipose tissue of ruminants.

In rhesus monkeys the fatty acid composition of erythrocytes is influenced by the content of fatty acids in the diet (Fitch et al., 1961). It would be anticipated that in leaf-eating monkeys with microbial activity in their stomach the dietary polyunsaturated fatty acids would be hydrogenated and thus would be less efficiently incorporated into plasma and erythrocytes. By feeding pellets with soybean oil or linseed oil, which are rich in linoleic and α-linolenic acid respectively, to colobine monkeys and non-leaf-eating primates (monogastric primates) and comparing their fatty acid patterns of blood plasma and erythrocytes we tried to find an indication of microbial hydrogenation of the two fatty acids the stomach of colobines.

Materials and methods

Animals and experimental design

During annual routine vaccination at Rotterdam Zoo in 2003 and 2004 blood samples were obtained from François langurs (*Trachypithecus françoisi*), Javan langurs (*Trachypithecus auratus auratus*), black and white colobus (*Colobus* g*uereza*), gorillas (*Gorilla gorilla*) and a white-handed gibbon (*Hylobatus lar*). In addition to their regular produce, fruits and browse, the monogastric primates received a primate pellet which contained soybean oil as major fat source (PP-SBO) and a langur pellet also with soybean oil (LP-SBO). Equal amounts of PP-SBO and LP-SBO were supplied but the actual consumption of each product is not known. The leaf-eating primates received the langur pellet with soybean oil as major fat source (LP-SBO) followed by the same langur pellet but containing linseed oil (LP-LSO) instead of soybean oil. All pellets were manufactured by Arie Blok Diervoeding, Woerden, The Netherlands.

Blood samples were collected according to the schedule given in Table 1. Blood samples were taken from the jugular vein into EDTA-containing vacuum tubes. The samples were centrifuged at 2700 x g for 5 minutes to separate plasma from the cells. The buffy coat was removed and the erythrocytes were washed twice with cold, demineralised water. The plasma and erythrocyte samples were stored at –80 ^{O}C until analyses. Fatty acid composition of plasma and erythrocytes was determined as described below.

Table 1. Trial schedule

Experiment	Period	Animals	Pellet	Fatty acids plasma	Fatty acids erythrocytes
A	November, December 2003, January 2004	4 François langurs	LP-SBO	4x	2x
		4 Javan Langurs		4x	1x
		3 Black and white colobus		3x	1x
B	October, November 2004	3 François langurs	LP-SBO	2x	3x
		4 Javan Langurs		3x	4x
C	2003, 2004	4 Gorillas	PP-SBO + LP-SBO	4x	None None
		1 Gibbon	PP-SBO	1x	

Chemical analyses

Dry matter, crude ash, crude protein, crude fat and crude fibre were analysed according to the Weende analyses (AOAC 1984), and neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin (ADL) according to Van Soest (1992).

For fatty acid analyses feedstuffs and plasma were extracted with chloroform-methanol (2:1, v/v) according to the procedure described by Folch et al. (1957), and the extracts were saponified and methylated as described by Metcalfe et al. (1966).

Table 2. Composition and the fatty acid patterns of the concentrates. The outcome of Weende and Van Soest analyses are expressed as % of the dry matter

	Langur pellet with soybean oil (LP-SBO)	Langur pellet with linseed oil (LP-LSO)	Primate pellet with soybean oil (PP-SBO)
Used in experiment	A and C	B	C
Dry matter	93.9	94.2	90.8
Ash	4.7	3.5	5.5
Crude protein	25.6	22.5	18.5
Crude fibre	28.0	36.9	2.7
Crude fat	6.9	3.1	6.7
NDF	44.0	62.6	11.1
ADF	31.3	44.8	2.7
Lignin	4.6	9.3	0.6
Fatty acids (%)[1]			
C14:0	0.2	0.2	0.9
C16:0	12.9	9.6	27.2
C16:1	0.2	-[2]	0.5
C18:0	3.4	4.7	3.2
C18:1 n-9	24.3	26.7	28.1
C18:1 n-7	1.7	1.1	1.2
C18:2 n-6	49.0	20.8	31.2
C18:3 n-3	4.9	26.8	2.4
C20:0	0.4	0.4	0.3
C20:1 n-9	0.4	0.4	0.8
C22:0	0.5	0.3	0.2
C22:1 n-9	-	0.7	-
C24:0	0.3	0.3	0.2
Unknown		7.0	
Others	1.8	1.3	3.8

[1] Fatty acid values are expressed as g fatty acid methyl ester/100 g fatty acid methyl esters. Fatty acids are presented in shorthand notation: number of carbon atoms followed by number of double bonds and incidentally place of double bond.
[2] Limit of detection is approximately 0.2%.

Table 3. Main fatty acid in blood plasma and erythrocytes of langurs and non-leaf-eating primates on a diet with soybean oil and linseed oil, experiment A, B and C

Fatty acids[1]	Langurs (A)		Langurs (B)		Primates (C)
	Plasma	Erythrocytes	Plasma	Erythrocytes	Plasma
	n = 11	n = 4	n = 5	n = 7	n = 5
C16:0	21.3 ± 3.1	28.9 ± 3.4	19.6 ± 3.7	27.2 ± 3.0	25.5 ± 5.1
C16:1	1.8 ± 0.6	0.6 ± 0.2	1.6 ± 0.5	0.4 ± 0.3	2.2 ± 0.7
C18:0	12.6 ± 2.9	16.7 ± 1.7	13.4 ± 1.5	18.0 ± 2.2	6.8 ± 0.6
C18:1 n-9	18.3 ± 3.1	9.5 ± 1.1	16.9 ± 3.6	8.8 ± 1.2	21.2 ± 6.0
C18:1 n-7	2.6 ± 1.2	2.6 ± 1.5	2.4 ± 1.0	2.0 ± 0.6	2.6 ± 0.9
C18:2 n-6	20.7 ± 5.2	7.2 ± 1.4	17.3 ± 4.4	6.4 ± 1.1	24.2 ± 11.5
C18:3 n-3	2.0 ± 1.0	0.4 ± 0.5	4.6 ± 0.6	0.8 ± 0.1	0.8 ± 0.6
C20:3 n-6	2.6 ± 1.0	1.2 ± 0.5	3.0 ± 0.8	1.4 ± 0.5	0.9 ± 0.4
C20:4 n-6	5.4 ± 1.5	7.8 ± 3.7	6.3 ± 1.0	10.2 ± 3.6	6.6 ± 5.0
C20:5 n-3	0.8 ± 0.6	0.3 ± 0.6	1.7 ± 0.4	0.8 ± 0.7	-[2]
C22:4 n-6	0.3 ± 0.6	0.6 ± 0.4	-	0.6 ± 0.5	0.4 ± 0.4
C22:3	-	3.5 ± 1.4	-	3.6 ± 2.3	0.4 ± 0.5
C22:5 n-3	0.8 ± 0.5	1.0 ± 0.8	1.3 ± 0.2	1.6 ± 0.7	0.6 ± 0.1
C24:0	-	5.3 ± 1.2	-	4.9 ± 0.5	-
C22:6 n-3	1.1 ± 1.1	0.4 ± 0.4	1.2 ± 0.4	0.5 ± 0.6	2.5 ± 1.4
C24:1	-	1.9 ± 0.3	-	1.7 ± 0.4	-

[1] See note 1, table 2.
[2] Limit of detection is approximately 0.4 %

Table 4. Contents of stearic and oleic acid isomers relative to the n-6 and n-3 fatty acids in feed pellets, plasma and erythrocytes of the langurs and the gibbon.

	Pellet	Plasma	Erythrocytes
Pellet: **LP-SBO**		N = 11	N = 4
C18:0/sum C18:1	0.1	0.6 ± 0.2	1.4 ± 0.5
C18:0/n-6 family	0.1	0.4 ± 0.1	1.1 ± 0.4
Sum C18:1/n-6 family	0.5	0.7 ± 0.2	0.7 ± 0.2
Pellet: **LP-LSO**		N = 5	N = 7
C18:0/sum C18:1	0.2	0.7 ± 0.2	1.7 ± 0.3
C18:0/n-3 family	0.2	1.5 ± 0.2	6.6 ± 4.7
Sum C18:1/n-3 family	1.0	2.2 ± 0.5	3.8 ± 2.4
Pellet: **PP-SBO**		N = 1	
C18:0/sum C18:1	0.1	0.2	
C18:0/n-6 family	0.1	0.3	
Sum C18:1/n-6 family	0.9	1.5	

The fatty acid methyl esters were separated and quantified by gas liquid chromatography. Erythrocytes were mixed with isopropanol and the lipids were extracted with hexane containing butyl-hydroxyl-toluene (Nelson,1975). Methylation of erythrocyte fatty acids was performed according to the method of Angelico et al. (1983), and the fatty acid profile was determined using gas liquid chromatography.

Results

Table 2 shows the analysed composition of the pellets used. Crude protein was highest in LP-SBO, whereas it was lowest in PP-SBO. Crude fibre, NDF and ADF were considerably higher in LP-LSO than in LP-SBO and PP-SBO. The percentage fat was similar in LP-SBO and PP-SBO and was twice as high as that in LP-LSO. The greatest differences in fatty acid composition were found in the contents of palmitic (C16:0), oleic (C18:1 n-9), linoleic (C18:2 n-6) and α-linolenic acid (C18:3 n-3).

The fatty acid compositions of plasma and erythrocytes in the langurs and non-leaf-eating primates for the different experiments (Table 1) are presented in Table 3. After switching the langurs from LP-SBO to LP-LSO, the content of α-linolenic acid (C18:3 n-3) rose for both plasma and erythrocytes. There was considerable within-group variation in the values for C18:2 n-6 (linoleic acid) and alpa-linolenic acid in the erythrocytes of the non-leaf-eating primates, but it would appear that the values were not higher than those in the langurs.

Discussion

The main differences in the fatty acid patterns of the two types of pellets fed to the langurs (LP-SBO and LP-LSO) lies in the contents of palmitic, linoleic and α-linolenic acid. However, the pellet that was fed to the non-leaf-eating primates (PP-SBO) differed considerably from PL-SBO, especially concerning palmitic and linoleic acid. It is unknown in which proportion the two types of pellets were consumed by the langurs and gorillas. The gibbon was only fed with the primate pellet (PP-SBO).

The plasma fatty acids are present in cholesteryl esters, triacylglycerols, phopholipids and non-esterified fatty acids, but the fatty acid composition of plasma determined here lumps all the fractions together. Fatty acids in erythrocytes are part of the structural lipids in the membranes. From Table 3 it can be concluded that the contents of linoleic and α-linolenic acid in erythrocytes was much lower than in plasma, but the erythrocytes had a considerable content C 23:3 and C 24:0.

At first sight, the results may be interpreted that there is no significant hydrogenation of linoleic and α-linolenic acid in the stomach of langurs. After feeding LP-LSO instead of LP-SBO there was a clear increase in the content of α-α-linolenic acid in both plasma and erythrocytes. Furthermore, the langurs did not have lower content of α-linolenic acid in erythrocytes than did the gorillas, even though the latter monkeys had higher group levels of linoleic acid.

It could be argued that the above interpretation of the data is too simplistic. The C18 polyunsaturated fatty acids can undergo chain elongation as well as desaturation. The products of hydrogenation of the C 18 polyunsaturated fatty acids can be various isomers of C18:1 and ultimately C18:0 (stearic acid). Therefore we have looked at the proportions of the possible products of hydrogenation relative to all fatty acids of the n-6 family for LP-SBO and at those relative to all fatty acids n-3 family for LP-LSO. The results are presented in Table 4. On both diets, but especially on the diet with LP-LSO one can see a shift to more saturated fatty acids in plasma and erythrocytes. Especially stearic and oleic acid isomers were increased when compared to polyunsaturated fatty acids in the erythrocytes. This could be interpreted as an indication that microbial hydrogenation takes place before absorption of the fatty acids. However, this interpretation is toned by the fact that the novo synthesis of C18:0 and C18:1 is neglected, which is incorrect, and by lack of information on quantitative data and on the time course of the various ratios. Calculations for the non-leaf-eating primates are much less reliable than those for the langurs because of the two types of pellets with a different content of linoleic acid. However, the gibbon consumed only the primate pellet and therefore we can make the same calculation. Here there is also a tendency that the more saturated fatty acids in plasma are increased relatively, but it concerns only one animal.

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CHAPTER 11

Effect of dietary fibre on the faeces score in colobine monkeys at Dutch Zoos

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Abstract

In captivity colobine monkeys often display a soft to watery faecal consistency, in contrast to their wild conspecifics, which usually display well-formed faeces. It has been suggested that dietary fibre, and possibly also dietary water content is related to faecal consistency. To further test this assumption we pooled data on 15 individual feeding periods from six feeding trials with colobine monkeys from different species, during which dietary and faecal chemical composition had been determined and faeces consistency had been scored with one consistent scoring scheme. Our pooled data suggest that dietary neutral detergent fibre (NDF) and dietary dry matter content were significantly, positively correlated to a better faeces consistency, whereas dietary protein content was negatively correlated to faeces consistency. Influences on the faeces consistency like easy digestible carbohydrates and the mineral content of the diet were not considered. Firmer faeces did not contain less water, but more NDF and less protein. It is suggested that diets fed to colobine monkeys in Dutch zoos should be reduced in protein and dietary water, and increased in fibre content. A recognized problem for attaining this goal is the lack of a readily accepted primate pellet that is not only called “high fibre” but that actually mimics the fibre content of the diet of free-ranging colobine monkeys.

Introduction

Leaf-eating primates have been recognized for a long time as primates which are difficult to adapt to captive diets (Edwards 1995, NRC 2003). These primates are foregut-fermenters, with a multi-chambered forestomach where plant material is fermented by symbiotic gut bacteria, but also with a well-developed hindgut in which additional fermentation occurs (Bauchop and Martucci 1968, Caton 1999). A high incidence of gastrointestinal disorders has been observed repeatedly in these primates and been linked to the feeding of rapidly fermentable foods such as commercial fruits and vegetables (Hill 1964, Bauchop and Martucci 1968, Hölihn 1973, Taff and Dolhinow 1989, Janssen 1994, Nijboer and Dierenfeld 1996). In spite of very strict feeding recommendations that advise a feeding regime of a high-fibre primate pellet, green leafy vegetables, and browse (Edwards et al. 1997), these animals are still often fed on diets containing fruits, coloured vegetables, milk products, and pellets low in fibre but high in protein (Nijboer and Dierenfeld 1996). In part, this discrepancy stems from the misconception that animals termed, at least occasionally, “frugivores”, should receive fruits in a captive environment. However, there is a well-known discrepancy between the chemical composition of “wild” and “cultured” fruits and vegetables; for example, Conklin-Brittain and Wrangham (2000) compared the composition of wild ripe fruits, domesticated fruits and vegetables, wild young leaves and domesticated greens in Africa eaten by primates (Table 1). Their data clearly show that cultured produce is drastically higher in sugars and drastically lower in fibre, as measured by neutral detergent fibre (NDF) or acid detergent fibre (ADF). The values for the food items ingested by free-ranging colobine monkeys reviewed by Nijboer and Dierenfeld (1996) and Lawrence et al. (2005) were similar to those for “wild” food items (Table 1).

A common problem in captive colobines is the production of unformed faeces of a “pie” consistency which contrasts to the usually well-formed, firm faeces these animals produce in the wild (Nadler 1994). In domestic animals, unformed faeces are a sign of gastrointestinal upset of various aetiologies; malfermentation in either the fore- or hindgut, due to the presence of too much rapidly fermentable carbohydrates, leading to an acid overload (rumen or caecum acidosis), or an excessive provision of protein, leading to smeary feaces in dogs (Zentek et al. 2004). In a recent report, we even found a statistically significant correlation between the dietary water intake and faeces consistency in a group of captive colobines and suggested that an excessive water intake, due to a diet based on fruits and vegetables extremely low in dry matter content, could contribute to the problem of unformed faeces in colobines (Nijboer et al. 2006b). In order to address this problem with a larger dataset, we here combine data generated in different trials on colobine monkeys in The Netherlands on dietary and faecal nutrient composition and faeces consistency.

Table 1: Mean chemical composition of wild and cultured non-seed plants (Conklin-Brittain and Wrangham 2000) used by free-ranging primates and in captive primate husbandry, as well as the ranges for the whole diets of free-ranging Asian (Nijboer et al. 1997) and African (Lawrence et al. 2005) colobines (% in DM).

Food	N	DM	CP	SUG	NDF	ADF
Wild ripe fruits	50	27	11	16	37	26
Wild young leaves	56	27	26	5	42	28
Cultured fruits	26	17	6	40	14	9
Cultured vegetables	18	10	17	29	18	14
Cultured greens	13	9	23	25	19	14
Asian colobine diets	-*	-	13	-	44-57	31-52
African colobine diets	-	-	17	-	36-47	23-42

N number of samples, DM dry matter (% fresh weight), CP crude protein, SUG free sugar, NDF neutral detergent fibre, ADF acid detergent fibre (all % dry matter)
- = not given.

Methods

All trials from which data were used followed a similar design: the diet offered and consumed to a group of monkeys was recorded, faeces of the whole group were scored for consistency and collected in total, and representative samples of diet and faeces were analysed for nutrient content. Between one and three diets were fed per trial. The following langurs were used for the comparison of the diets and faecal scores:

Trial 1a-c: Four François langurs (*Trachypithecus françoisi*) at Rotterdam Zoo (Nijboer et al. 2001).

Trial 2a-b: Three spectacled leaf monkeys (*Trachypithecus obscurus*) at Artis Zoo (Nijboer et al. 2006c).

Trial 3: Five black and white colobus monkeys (*Colobus guereza*) at Rotterdam Zoo (Nijboer et al. 2006c).
Trial 4a-c: Two adult Javan langurs (*Trachypithecus auratus auratus*) at Rotterdam Zoo (Nijboer et al. 2006b).
Trial 5a-c: Four adult , four sub adults and two juvenile Javan langurs (*Trachypithecus auratus auratus*) at Apenheul Zoo (Nijboer et al. 2006a).
Trial 6a-c: Three females, two males and three adults, a sub adult and a juvenile Javan langurs (*Trachypithecus auratus auratus*) at Rotterdam Zoo (Nijboer et al. 2006a).

Here, the dry matter (DM, % fresh weight), crude protein (CP, %DM), neutral detergent fibre (NDF, %DM) and acid detergent fibre (ADF, %DM) content of the diets ingested and the faeces voided during the trial periods, are reported. Additionally, the mean faecal score (FS) of each trial period, scored using the system developed by Waltham (2000) and adjusted for langurs by Nijboer (2006a), are presented. Mean FS score was calculated as: (score 1 x number + score 2 x number + score 3 x number + score 4 x number + score 5 x number)/total number. The score ranges from 1-5, with 2 considered as representative for free-ranging colobines, and 5 corresponding with watery diarrhoea.

Regression analysis served to evaluate the relationship between NDF, the water content of the diets and the score of the faeces. For regression analysis we used 15 data sets which were assumed to be independent. The significance level was set at $P < 0.05$.

Results

The nutrient composition of the diets ingested by the animals, of the faeces, and the mean FS per diet period are summarized in Table 2. The faeces had a consistency ranging for 1 to 5 which were both observed during the trials. During no trial period a mean FS of 2 (the assumed normal) was attained.
The mean crude protein content of the captive diets (18.9 %DM) was high compared to the ranges for the whole diet of free ranging colobines (Table 1). The mean NDF (36.7 %DM) and ADF (25.4 %DM) contents of the diets used were relatively low. Linear relationships between dietary or faecal parameters and FS are summarized in Table 3.

Table 2: Dietary and faecal composition (% in DM), and mean faecal consistency, in different trials with captive colobine monkeys (see Methods).

Trial	-------- Diet --------				-------- Faeces --------				FS
	DM	CP	NDF	ADF	DM	CP	NDF	ADF	
1a	16.4	19.9	41.1	27.7	22.8	24.1	53.4	13.8	3.5
1b	23.0	20.0	51.6	34.7	26.9	17.9	55.2	14.9	2.6
1c	21.2	20.4	41.4	27.1	22.9	23.4	51.1	36.2	3.2
2a	15.4	19.9	28.7	22.6	26.8	22.6	45.4	36.8	3.8
2b	22.0	20.8	26.6	11.7	22.0	34.5	39.8	29.0	4.1
3	21.8	19.4	43.4	31.8	37.0	24.9	51.4	40.8	3.1
4a	12.3	18.7	36.7	27.3	24.4	19.5	51.5	37.8	3.0
4b	24.7	13.7	35.8	30.3	26.7	19.9	50.9	39.8	2.7
4c	17.4	16.6	34.8	27.2	22.2	20.0	50.1	39.1	3.3
5a	16.8	20.3	31.9	20.3	-*	-	-	-	3.6
5b	46.6	17.8	30.0	23.6	-	-	-	-	2.6
5c	19.1	18.5	35.7	26.9	-	-	-	-	3.2
6a	17.3	19.5	34.4	20.8	-	-	-	-	3.4
6b	36.6	19.1	41.0	25.0	-	-	-	-	2.6
6c	24.4	18.7	37.3	23.5	-	-	-	-	3.4

DM dry matter (% fresh weight), CP crude protein, NDF neutral detergent fibre, ADF acid detergent fibre (all % dry matter), FS faecal score (on a scale from 1-5)
- = not given

Table 3: Linear relationships between dietary or faecal parameters and the mean faecal score (FS) in the dataset of Table 2, according to the equation FS = C + β *parameter.

Parameter	C	β	Adjusted R^2	P-value
Dietary				
DM	3.88 (± 0.28)	-0.03 (± 0.01)	0.284	0.024
CP	0.94 (±1.16)	+0.12 (± 0.06)	0.167	0.073
NDF	4.64 (± 0.60)	-0.04 (± 0.02)	0.254	0.032
ADF	4.64 (± 0.43)	-0.06 (± 0.02)	0.427	0.005
Cellulose	4.33 (± 0.31)	-0.06 (± 0.02)	0.477	0.003
Fecal				
DM	4.20 (± 0.95)	-0.04 (± 0.04)	0	0.356
CP	1.51 (± 0.53)	+0.08 (± 0.03)	0.564	0.012
NDF	7.49 (± 1.15)	-0.09 (± 0.02)	0.612	0.008
ADF	3.32 (± 0.58)	-0.002 (± 0.02)	0	0.941

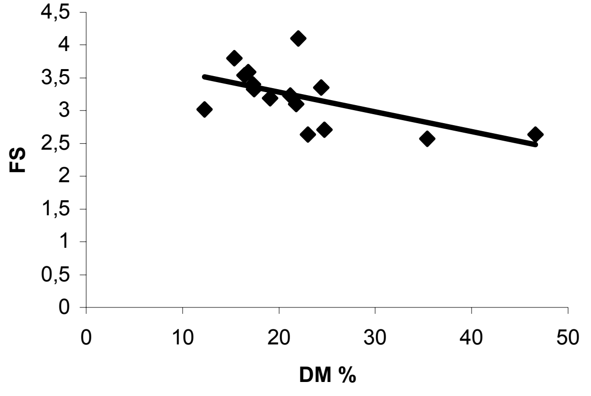

Figure 1. Correlation between dietary dry matter content (DM in % fresh weight) and faecal score (FS) in feeding trials with captive langurs.

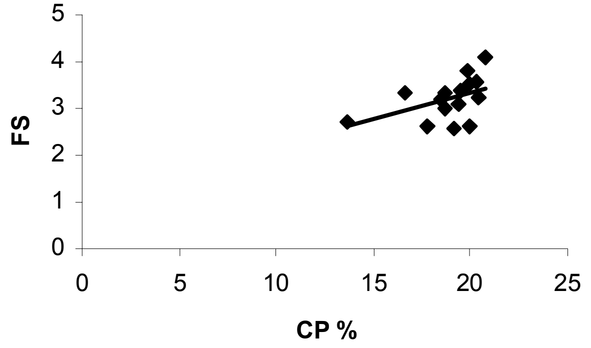

Figure 2. Correlation between dietary crude protein content (CP in % DM) and faecal score (FS) in feeding trials with captive langurs.

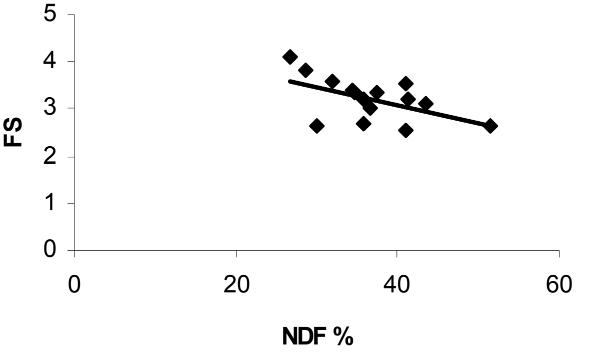

Figure 3. Correlation between dietary neutral detergent fibre content (NDF in % DM) and faecal score (FS) in feeding trials with captive langurs.

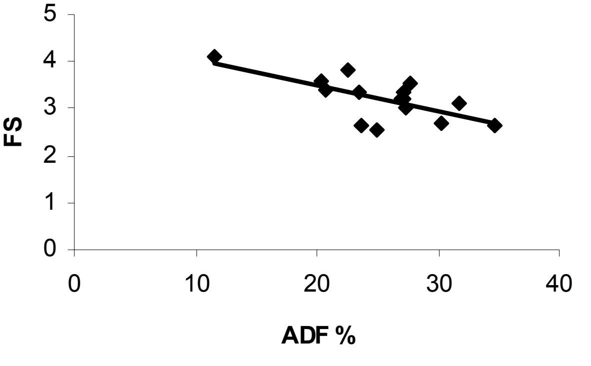

Figure 4. Correlation between dietary acid detergent fibre content (ADF in % DM) and faecal score (FS) in captive langurs.

Among the dietary parameters, DM (Fig. 1), CP (Fig. 2), NDF (Fig. 3), and ADF (Fig. 4) content were all correlated to FS, although the correlation with CP only tended towards significance ($p=0.073$).
Multiple and linear regression analysis were applied to evaluate:
FS = 3.380 (± 0.708) - 0.026 (± 0.007)* DM + 0.105 (± 0.032)*
CP - 0.046(± 0.009)*NDF (DM, CP, NDF in % DM; adjusted R^2 =0.782; $P=<0.001$).
Among the faecal parameters, CP but not DM was correlated to FS. Most importantly, there was a high correlation between faecal NDF, but not ADF, with FS.

Discussion

The results confirm that dietary nutrient composition is a decisive factor for the faecal consistency in captive colobines. The faecal consistency rarely is a target of research in domestic animal nutrition, with the exception of pet animals such as dogs or rabbits. In the carnivorous dog, the addition of fibre will improve faecal consistency (Sunvold et al. 1995), and diets containing certain protein sources can lead to maldigestion in the hindgut with an increase in proteolytic bacteria such as *Clostridium spp*., resulting in smeary faeces (Zentek et al. 2004). In pet rabbits, smeary faeces are a common finding in animals fed a diet particularly low in fibre (Quesenberry and Carpenter 2004). In general, as confirmed in this study in colobines, increasing dietary fibre levels will improve faecal consistency (Nijboer et al. 2006b). Constipation due to high amount of short fibre particles has never been recorded although constipation in the GIT can occur if long stripes of bark are ingested (Calle et al. 1995).

Faeces consistency is not a parameter of proven, direct physiological relevance. To our knowledge, there are no published studies linking faeces consistency as such to other health parameters, such as longevity, body condition, immune or reproductive status. However, faeces consistency is the parameter most easily measured in captive wild animals, and the first one noted by zoo keepers, and therefore a useful tool for the detection of change. Given that it is the aim of captive management of a particular species to maintain the animals in the physiological state that they have adapted to throughout evolution, it is desirable for zoos to achieve a faeces consistency in their animals that resembles that in free-ranging animals. In the case of colobines, the results of this analysis suggest that diets higher in fibre, and maybe also lower in water and protein content than those conventionally used in Dutch (and probably other European) zoos would promote firm faeces in colobines (Figure 1, 2, 3 and 4). Firm faeces could be interpreted as indicators of a normal digestive physiology typical for the species. However, the results have been obtained from 15 data and no information is available if the correlation is prolonged outside the range of the observed data. In the outline of this project correlation between mineral content and easy digestible carbohydrates of the diet and faeces consistency were excluded.

Table 4: Fibre levels in commercially available primate products, manufacturers' information in the guaranteed analysis panel was re-calculated to a dry matter basis.

Diet name	Crude fibre (% dry matter)	NDF (% dry matter)
Leaf-Eater Primate diet[1]	15.6	27.4
Primate High Fiber Sticks[1]	16.1	32.9
Primate Browse Biscuit[1]	17.8	29.4
Leaf Eater Primate[2]	12.4	21.3
High fiber primate diet[3]	10.0	-
Leaf Eater Red Apple[4]	14.4	20.8
Langur pellet[5]	28.0	44.0

[1] Mazuri® (PMI, St. Louis, MO USA)
[2] MazuriTM (SDS, Essex, UK)
[3] HMS (Bluffton, IN USA)
[4] Marion Zoological (Plymouth, MN USA)
[5] Hope Farms (Woerden, The Netherlands)

A major problem in the feeding of primates is the discrepancy between the fibre content of truly foli- and frugivorous species (Table 1) and the fibre levels in primate pellets commercially available (Table 4). Even if many of these products are named "high fibre" or "leaf eater", their fibre levels are distinctively lower than those of the free-ranging diets of folivorous primates. Given the fact that such pellets are mostly supplemented with diet items of even lower fibre content, they appear not suitable to guarantee a total diet fibre content similar to the wild.

Although Edwards (1997) promoted the use of a manufactured diet of very high fibre content – with an ADF level of 30 % -, such a diet does not seem to be in widespread use in Europe. Note that in order to achieve an ADF level of 30 % DM, an NDF level of at least 40 % DM or higher is warranted. Primates are unlikely to eagerly consume any manufactured feed of such a fibre content, and even less so if they had been used to other, more palatable food of lower fibre content for months or years before the diet change. As the success of a new diet is often measured by its acceptance by the animals, manufacturers might be reluctant to produce such high-fibre diets.

We have speculated already that an excessive dietary water intake could also influence faecal consistency in colobine monkeys (Nijboer et al. 2006b). Across all the trials used in this survey, there was an average dietary water intake of 752 ml per animal. As the mean body weight of the animals used was between 6.0 and 6.5 kg, one would, according to the allometric equation for daily water flux given by Robbins (1993), expect a daily water flux in these animals between 620-670 ml. Cultivated fruits, vegetables and greens contain a higher amount of water compared to the natural foods of these primates. Also according to personal observation no intake of water from a pan is observed when huge amount of cultured fruits, vegetables and greens are fed. Thus, it could be hypothesized that the high proportion of very water-rich food items leads to an excessive water intake with consequences on faecal consistency.

An interesting correlation found in this study was the one between both dietary and faecal protein content and faeces consistency. Although the mean dietary protein content of the diets used in the trials was higher than that reported for free-ranging colobines, the difference does not appear very drastic. However, one should note that crude protein data given for the diet of free-ranging animals, as that given in Table 1, is still likely to be an overestimate (Lawrence et al. 2005). Protein (N x 6.25) can be chemically bound (acid detergent insoluble nitrogen = ADIN) and cannot be digested in the rumen (Van Soest 1994), however no information is available if ADIN is digested in the gut. As analysed by Nijboer (1996) up to 50 % of the analysed crude protein in the diets of free-ranging lemurs can consist of ADIN and therefore be unavailable for “rumen” fermentation. Thus, mimicking such a calculated protein content with protein sources of completely available protein will lead to an oversupply of available protein. Whether such an oversupply is of any physiological consequence, or can be compensated by the animal, largely remains to be investigated in wild herbivore species. Moreover, it cannot be excluded that the observed effect of dietary protein on faecal score is not causal, but linked to other characteristic of the diet. However, the fact that protein did have an independent influence on faeces consistency could indicate that when designing diets for folivorous primates, moderate contents of available protein might be advisable.

Conclusions

1. In this set of data from colobine monkeys, faeces consistency is dependent on dietary water content (negative influence), dietary protein content (negative influence), and dietary fibre content (positive influence).

2. The observation that in captive animals, faeces of a softer consistency are observed than in free-ranging animals, corresponds to the fact that diets of captive individuals are often lower in fibre, and higher in available protein, than reported diets of free-ranging colobines.

3. Therefore, captive feeding regimes should strive to increase fibre content while keeping (available) protein content moderate.

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CHAPTER 12

GENERAL CONCLUSIONS

In this section the main conclusions from this thesis are listed and briefly discussed.

Chapter 2 The digestive physiology and colobine primates

A brief review was presented on the digestive physiology of langurs. According to the literature colobines have a compartmentalized stomach in which food is fermented. The structure and size of the hindgut suggests that here fermentation can also take place. In this chapter a schematical overview of the digestive tract was shown as well as the digestive tract collected from an euthanised langur. In langurs fermentation takes place by microbes in the fore stomach. High-starch or high sugar products can lead to a acidosis in the fore stomach.

Fiber fermentation is an important aspect in the physiology of langurs. Fiber can be divided into cell content and cell wall components. The cell wall contains lignin, ADF and NDF and a part of the pectic substances, galactans an ß-glucans. The cell contents can easily be feremented and thus apparently digested.

Chapter 3 Comparison of diets fed to Southeast Asian colobines in North American and European zoos, with emphasis on temperate browse composition

Results of a nutrition questionnaire that was sent to North American and European facilities showed that there is currently no standardized diet fed to Southeast Asian colobines kept in captivity: one hundred and forty nine different kinds of foodstuffs (green, vegetables, browse, fruits, animal products and concentrates) were fed. European zoos feed a greater diversity of fruits and vegetables compared with North American zoos. Diets fed in captivity contained less fiber and more protein than in the natural foods.

However, the amount of browse was variable in zoos being an essential part of the diet. Elm, maple, poplar, willow, rose leaves and twigs were most frequently fed in the northern hemisphere. Trees contained fiber and protein levels within ranges reported for native foods. Rose leaves have less fiber and more protein and are less suitable. The leaf:twig ratio of browse in September samples was less than in the July samples. The samples in the later growing season contained more NDF and ADF.

Chapter 4 Chemical composition of Southeast Asian colobine foods.

In the wild the food choice of Asian colobines varies seasonally. Available fruits and seeds are a frequent component of the diet. If these products are not available the langurs switch to eating leaves.

Summarizing nutrition data of the food from the wild of three langur species showed that the available protein approaches the minimum required for the maintenance of microbial populations in ruminants. Chemical analyses of wild fruits, seeds and leaves showed that they were characterized by high levels of NDF and indigestible fibre (lignin). It is suggested that seeds and fruits are a variable source of dietary energy. Calcium content of flowers and seeds was higher than in many leaf samples. In contrast to cultivated seeds and nuts which contained mostly low amounts of calcium. Based on the food items consumed in the wild, ruminant dietary requirements may provide a suitable guideline.

Chapter 5 Chemical analyses and consistency of faeces produced by captive François monkeys (François langurs, *Trachypithecus françoisi*) fed supplemental fibre.

Langurs prefer a diet containing carbohydrates which are easily digested, but when such a diet is not available, langurs consume diets with more fibre and less digestible carbohydrates. This study shows an improvement of stool quality when a high fiber pellet was fed to François langurs instead of a low fibre pellet. The dietary water intake was lower than with the control diet. The faeces contained slightly less water, and a lower amount of fibre and more non-structural carbohydrates. It was suggested that the non-structural carbohydrates had a superior water-binding capacity. The apparent digestibility of crude fibre increased when the high fibre diet was fed instead of the control diet.

Chapter 6 The influence of introducing a high fiber pellet on the time budget of captive François langurs (*Trachypithecus françoisi*)

Feeding a high fibre diet, which is less palatable than a low fibre diet did not significantly influence the behaviour of François langurs. The total time spending on feeding, resting, moving and grooming did not change and no more aggressive behaviour was seen. Feeding a high fibre pellet resulted in less distinct feeding peaks and eating was distributed more over the whole day.

Chapter 7 Macronutrient digestibility and faeces quality in captive black and white colobus (*Colobus guereza*) and captive spectacled leaf monkeys (*Trachypithecus obscurus*)

Experiments with captive black and white colobus and spectacled leaf monkeys showed that faeces quality improved for these foregut fermenting primates when feeding more crude fiber and cellulose.

The control diet for both species contained browse, pellets, greens, fruits and vegetables, but the experimental diet of the captive spectacled leaf monkeys was restricted in greens, fruits and vegetables. Soft faeces and diarrhoea was frequently seen in diets with low amounts of fibre and high amounts of nitrogen free extract. This was demonstrated with the spectacled leaf monkey diet during the control period. The experimental diet of the spectacled leaf langurs contained more fibre which resulted in improved faeces consistency. The diet of the black and white langurs contained more crude fibre and cellulose than the diets of the spectacled leaf monkey, which resulted in a superior faeces quality.

Free ranging leaf-eating langurs spent a large part of the day on foraging. This strategy of dispersing feeding bouts over time reduces the sudden influx of rapidly fermentable material. Therefore it was suggested to feed captive animals at least 4 times a day.

Chapter 8 Effect of diet on the feces quality in Javan langurs (*Trachypithecus auratus auratus*)

In two groups of Javan langurs in two facilities the effect of removal of produce from the diet was tested. Results in both facilities showed that the faeces quality improved when the diet consisted only of browse and a high fiber pellet. A correlation was found between a firmer feces consistency and an increase in NDF and a decrease in dietary water and a decrease in the intake of soluble sugars. High sugar content may lead to a drop in pH in the forestomach and a disruption of the normal symbiotic microflora which may result in gastric upset and chronic and intermittent diarrhoea as observed in ruminants with acidosis.

Dietary water may be a limiting factor. The water intake surpassed the daily water flux due to the high amount of produce. Although water intake was expected to be excreted in urine, it could be speculated that these high water intakes overcharged the capacity for gastrointestinal water absorption.

Chapter 9 Influence of two different diets on fluid and particle retention time in Javan langurs (*Trachypithecus auratus auratus*)

Tests were performed with two Javan langurs. During the control period they received a diet containing produce, browse and a high fiber pellet. During the test period the produce was left out which resulted in a higher intake of pellets and browse.

Exclusion of feeds rich in soluble carbohydrates (produce) caused an increased intake of high fiber pellets and browse. This resulted in a higher fiber intake and a lower dietary water intake and an improvement of the faeces quality. Apparently crude fiber, digestion was lower in the test period. Similar observations are found in many animal species which consume high amounts of fiber.

Mean Retention Time (MRT) was calculated by analysing the amount of cobalt-EDTA and chromium oxide in the food consumed and the faeces produced. No significant difference was found between the retention time of the fluid and particle part in both diets although the MRT increased in the period when the produce was left out. The dietary water intake did not exceed the water intake flux during that period. An increasing amount of fiber resulted in a decreasing of the passage rate and in a firmer faeces.

Chapter 10 **Comparison of the fatty acid patterns of blood plasma and erythrocytes in leaf-eating and non-leaf-eating primates fed diets with different fat sources**

Fatty acid composition of plasma and erythrocytes in langur blood was compared after feeding a diet rich in α-linolenic acid as opposed to a diet containing linoleic acid by comparing the values with those in gorillas and gibbons. After feeding the langurs with the diet rich in α-linolenic acid instead of that rich in linoleic, there was a clear increase in the content of alpha-linolenic acid in both plasma and erythrocytes. It indicates that dietary polyunsaturated fatty acids are hydrogenated in the stomach like they are in the rumen of ruminants. Similar conclusions could not be drawn for the blood values found in the blood of gorillas and gibbons.

Chapter 11 Effect of dietary fibre on the faeces score in colobine monkeys at Dutch Zoos

Comparison of 11 diets fed in Dutch zoos showed that faeces consistency depends on dietary water, protein content and fibre content. High water and protein intakes were associated with deteriorated faeces consistency, as where high fibre intake was associated with improved faeces quality.

In relation to the faeces quality, captive langur diets should have a higher dry matter and fibre content and a lower protein content when compared to free-ranging diets.

Summary

The research of this thesis is focussed on the intake of fibre and the effects of it on the faeces quality in leaf-eating primates.

In order to study the effects of the fibres more information is needed about the digestive physiology of foregut fermenting leaf-eating primates or langurs. Comparing the digestive system between foregut leaf-eating primates and ruminants show that there are similarities (chapter 2). Both groups have a foregut in which high quantities of fibres can be digested. Ruminants are normally eating grass however leaf-eating primates are specialised in eating leaves, greens and fruits. In both groups an excess of easy digestible carbohydrates can result in rumen acidosis. Clinical symptoms are a bloated rumen, weight loss and gastro intestinal problems.

Analysis of food consumed by langurs in North American and European zoos (chapter 3) show that many kind of different products are fed varying from apples, onion, green pepper, eggs, fennel to rose leaves. No advised standard diet is available for langurs in captivity. In general the diets contain much water and protein and less fibre like NDF, ADF and lignin. It is frequently seen it results is soft faeces and a poor body condition. As replacement of the natural leaves and twigs in western zoos leaves and twigs are fed from the trees in that area. The amounts NDF, ADF and protein are comparable with the wild consumed browse.

Analysis from the natural foods of three langur species show that fruits, seeds and leaves contain high amounts of protein, NDF, ADF and lignin. A part of the protein is not digestible in the rumen. Leaves can contain more than 50% NDF. Lignification in natural food is higher compared to the food fed in captivity (chapter 4).

When a diet with a high amount of fibre is fed, the faeces quality improves and is less soft (chaper 5). By increasing the amount of NDF with 10% and ADF with 7% in the diet of captive langurs the consitency of the faeces of François langurs increases with 40% and the consistency was similar as found in nature. During that period less greens and fruits and a pallet with higher amounts of NDF and ADF were fed.

A behaviour study performed during the tests described in chapter 6 shows that after supplying a diet with higher amounts of NDF and ADF no essential changing in the behaviour was observed. Average 20% of the time was spend on eating, 63 % on resting, 10% on moving, 10% on grooming and 0.33% on aggressive behaviour. The shown behaviour is comparable with the natural behaviour of white handed langurs which are similar to François langurs. However it was observed that pellets with high fibre was consumed more spread over the whole day.

The observed diet effect with François langurs are similar as seen in other leaf-eating primates (chapter 7). A clear link is observed between the consumed food for colobus primates and spectacled leaf langurs, the digestion and the faeces consistency. Tests with these leaf-eating primates showed that the faeces consistency increased when more crude fibre, NDF and ADF was fed. Especially the faeces of the spectacled langurs increased after increasing the amount of crude fibre and cellulose. Clearly was shown that the carbohydrate and crude fibre increased in the faeces when the diet contained more fibre. About 52-68% of the consumed crude fibre was digested during the tests with both species.

In chapter 8 the effect of deleting the greens and fruits from the diet of Javan langurs in Apeldoorn (Apenheul Zoo) and Rotterdam Zoo is described. This test consisted out of three periods. In the first and last period the diet contained greens and fruits whereas in the second period these products were delete from the diet. In the second period the diet consisted only out of high fibre pellets and leaves. That diet resulted in an increasing of the faeces consistency. Analysis showed that during the second period the intake of NDF increased whereas the intake of dietary water and easily digestible carbohydrate decreased. It is suggested that a langur diet should contain 46% NDF which results in an optimal faeces consistency compared to the faeces consistency found in the wild.

In chapter 9 a test is described with two Javan langurs. The retention time was measured in the normal diet which consisted out of a mixture of greens, fruits, pallets and leaves and with a test diet which consisted out of only pallets and leaves. Cobalt-EDTA and cromium oxide were used as indicator for respectively the fluid and particle retention time. During the test period the mean retention time increased for the fluid (47 hours) and the particle (49 hours) period, which were respectively 5 and 6 hours longer compared to the normal diet. The tests indicate that there is no significant differences between the fluid and particle mean retention time. From the diet and faeces analyses could be concluded that the passage rate decreased when the amount of dietary water and the amount of protein was lower; the amount of fibre increased. No correlation is found between the body weights and passage rate in foregut fermenting primates.

By hydrogenating of polyunsaturated fatty acids are converted in saturated fatty acids and geometrical and positional di- and mononoic isomers in the foregut of langurs (chapter 10). The results of the analyses of the serum and erythrocytes of langurs after feeding a high fibre pallet with soybean oil and linseed oil confirms that offered fatty acids are fermented in the foregut. In contrast no hydrogenating is found in monogastric primates in the foregut

In chapter 11 all consumed diets of the tested foregut fermenting primates are compared. Results from 11 diets of 6 foregut fermenting primate species of totally 29 primates were summarized and compared with the consistency of the excreted faeces during the test periods. Faeces consistency is negative influenced by the water content and the amount of protein an positive by the fibre content of the food.

Subsequently for their management in captivity it was concluded that an optimal diet for colobidae and particularly langurs in captivity should be based on the digestive strategy, the faeces quality and the composition and palatability of the foodstuffs.

Samenvatting

Het onderzoek in dit proefschrift heeft betrekking op de opname van celstof en het effect daarvan op de kwaliteit van de feces in bladetende apen.

Om de effecten van de voedingsvezels te bestuderen is inzicht in de vertering fysiologie van bladetende apen of langoeren noodzakelijk. Uit bestudering daarvan (Hoofdstuk 2) blijkt dat het verteringsysteem bij langoeren grote overeenkomst vertoont met dat van herkauwers. Beiden beschikken over een voormaag waarin grote hoeveelheden voedingsvezels kunnen worden verteerd. Herkauwers eten voornamelijk gras, terwijl langoeren vooral gespecialiseerd zijn in het eten van blad naast groente en fruit. In beide gevallen kan een overmaat aan gemakkelijk verteerbare koolhydraten leiden tot pens acidosis. Dat kan leiden tot klinische symptomen zoals een opgeblazen pens, gewichtsverlies en darmproblemen.

Uit analyses van het voedsel gevoerd aan langoeren in Noord Amerikaanse- en Europese dierentuinen (Hoofdstuk 3) blijkt dat gesteld kan worden dat er vele verschillende voeders worden gevoerd variërend van appels, uien, paprika, eieren, venkel tot rozen bladeren. Er bestaat dan ook geen standaard rantsoen. De rantsoenen bevatten in het algemeen veel water en eiwit en weinig voedingsvezels zoals NDF, ADF en lignine. Dit resulteert veelal in een matige kwaliteit feces en vaak een matige conditie van de dieren. Ter vervanging van de natuurlijk gegeten bladeren en takken in het wild worden in de Westerse dierentuinen de daar aanwezige bladeren en takken gevoerd. De NDF, ADF en eiwit samenstelling is vergelijkbaar met de bladeren en takken die in de natuur worden gegeten.

Uit analyses van het natuurlijk voedsel van een drietal langoeren blijkt (Hoofdstuk 4) dat het voedsel bestaande uit fruit, zaden en blad grote hoeveelheden eiwitten, NDF, ADF en lignine bevat. Een groot gedeelte van het eiwit is echter niet beschikbaar. Bladeren bevatten zelfs meer dan 50 % NDF. In verhouding tot in gevangenschap gevoerd voedsel is de lignificatie in de natuur veel groter. Zaden en fruit bevatten een gevarieerde hoeveelheid vet en kunnen een bijdrage leveren aan de energie voorziening.

Wanneer een rantsoen met een hoger vezel gehalte gevoerd wordt, verbetert de feces kwaliteit (Hoofdstuk 5). Bij een verhoging van het rantsoen met 10% NDF en 7% ADF van in gevangenschap gehouden François langoeren werd de feces 40% vaster van kwaliteit en was beter vergelijkbaar met de feces kwaliteit zoals die is waargenomen in de natuur. Er is in die periode minder groente en fruit gevoerd en een pellet met een hogere NDF en ADF fractie.

Door een gedragsstudie uit gevoerd bij François langoeren (Hoofdstuk 6) blijkt dat bij het verstrekken van een voeder met een hoger gehalte aan NDF en ADF geen wezenlijke veranderingen in de gedragingen van de dieren zijn waargenomen. Gemiddeld werd er 20% van de tijd besteed aan eten, 63% aan rusten, 10 % aan beweging, vlooien 10% en 0.33 % aan agressief gedrag. Dit gedrag komt overeen met het natuurlijk gedrag van de met François langoeren vergelijkbare withand langoeren. Wel werd waargenomen dat de brok met een hoog ruw celstof gehalte meer verspreid werd opgenomen over de dag.

Uit Hoofdstuk 7 blijkt dat de waargenomen resultaten bij de François langoeren ook gelden voor andere soorten langoeren. Er bestaat een duidelijk verband tussen het aangeboden voedsel van colobus apen en brillangoeren, de verteerbaarheid ervan en de kwaliteit van de feces. Bij proeven uitgevoerd bij brillangoeren en colobusapen blijkt dat de feces kwaliteit ook steviger wordt wanneer er meer ruwe celstof, NDF en ADF gevoerd wordt.Vooral bij de brillangoeren was de verbetering van de feces merkbaar toen er meer ruwe celstof en cellulose werd gevoerd. Ook was duidelijk aantoonbaar dat het koolhydraat en ruwe celstof gehalte in de feces hoger was bij een hoger vezel gehalte in het voer. Ongeveer 52-68 % van de opgenomen hoeveelheid ruwe celstof werd verteerd tijdens de proeven met de brillangoeren en colobus apen.

In hoofdstuk 8 staat het effect van het weglaten van groentes en fruit uit het rantsoen van mutslangoeren beschreven die gehouden werden in de dierentuin van Apeldoorn (Apenheul) en Rotterdam (Blijdorp). Tijdens een proef bestaande uit een drietal periodes werd het rantsoen met groentes en fruit gevoerd tijdens de eerste en derde periode. Tijdens de tweede periode bestond het rantsoen uit pallets en bladeren. In de tweede periode was de feces kwaliteit aanmerkelijk beter. Uit analyses bleek dat er meer NDF werd opgenomen en de hoeveelheid water opgenomen door het voer aanmerkelijk verminderde. Verondersteld wordt dan ook, dat een verlaagde opname van oplosbare suikers en water in het voer en een verhoogde opname van NDF tot een vastere feces lijdt bij langoeren. Uit de resultaten wordt gesuggereerd dat een gehalte van 46 % NDF in het voer lijdt tot een goede kwaliteit van de feces waarbij er geen verstoring van de vertering plaatsvindt.

In hoofdstuk 9 wordt een proef beschreven bij twee Javaanse langoeren. Bij beide langoeren werd de passage snelheid gemeten bij hun oorspronkelijk rantsoen bestaande uit een mengeling van groentes, fruit, pallets en bladeren. Vervolgens werd de passage snelheid gemeten bij hun rantsoen bestaande uit pallets en bladeren. Cobalt-EDTA en chroomoxide werden gebruikt als respectievelijk indicator voor de vloeibare and vaste fase. Gedurende het testrantsoen werd de gemiddelde passage snelheid (MRT) hoger. Deze bedroeg bij de vloeibare fase 47 uur en bij de vaste fase 49 uur, hetgeen respectievelijk 5 en 6 uur langer is dan in het oorspronkelijk rantsoen. Het verschil in passage tussen de vloeibare en vaste fase is klein. Uit de analyses blijkt dat de passage snelheid lager wordt wanneer de hoeveelheid water in het voedsel lage is en de hoeveelheid ruwe celstof in het voedsel hoger. Er bestaat geen verband tussen het lichaamsgewicht en passagesnelheid in voormaag verterende bladetende apen.

Door hydrolyse en biohydrogenering worden meervoudig onverzadigde vetzuren (PUFA), zoals linolzuur en linoleenzuur omgezet in verzadigde vetzuren zoals stearinezuur en geometrische isomeren. Wanneer in het oorspronkelijk rantsoen van de mutslangoeren de langoerenbrok met sojaolie vervangen wordt door een langoerenbrok met lijnolie blijkt dat de vetzuursamenstelling van het plasma en erytrocyten van die apen verandert (Hoofdstuk 10).

In Hoofdstuk 11 worden alle rantsoenen vergeleken van langoeren waarmee onderzoeken zijn uitgevoerd. Van 15 rantsoenen van 6 verschillende proeven is de samenstelling van het rantsoen vergeleken met de opgestelde feces score. Bij een hoger NDF en droge stof gehalte in het voedsel wordt de feces kwaliteit beter. Daarentegen wordt de feces kwaliteit slechter wanneer het eiwit gehalte lager is.

Langoeren of bladetende apen moeten dus een rantsoen hebben met een hoog droge stof en NDF gehalte en een normaal eiwit gehalte.

Samenvatting voor niet vakgenoten

Fokke en Sukke stelden vlot de diagnose toen ze ermee te maken kregen. Dit proefschrift houdt zich bezig met de kern van die diagnose maar kijkt ook naar de oorzaak van het probleem, echter niet bij mensen of eenden maar bij langoeren.

Fokke & Sukke: © Reid, Geleijnse & Van Tol

Langoeren zijn apensoorten met een heel ander darmstelsel dan bijvoorbeeld gorilla’s en chimpansees. Langoeren zijn apensoorten, die vooral in de tropische delen van Zuidoost Azië voorkomen. Hun voedsel bestaat vooral uit bladeren. Bladeren zijn door hun hoge vezelgehalte moeilijk te verteren. Langoeren kunnen dat wel, door hun specifiek darmstelsel. Ze hebben namelijk de beschikking over een maag die uit 3 of 4 delen bestaat en een darmstelsel waarin ook een gedeelte van de voedingsvezels kunnen worden afgebroken. De voedingsvezels in de natuur bestaan grotendeels uit moeilijk verteerbare delen zoals cellulose en lignine.

Door een enquête uitgevoerd bij dierentuinen in Noord Amerika en Europa die langoeren houden, blijkt dat men veel verschillende voedselsoorten voert. Deze voedselsoorten, groentes, fruit, bladeren en takken, bevatten veelal te gemakkelijk verteerbare koolhydraten zoals mono- en disachariden, zetmeel en hemicellulose. Het percentage cellulose in lignine is daarentegen veelal laag in verhouding tot wat in de natuur wordt gegeten door deze apensoorten (hoofdstuk 2 en 3).

Dit heeft tot gevolg is dat de langoeren in de dierentuinen regelmatig verteringsproblemen hebben, hetgeen resulteert in een slechte mestkwaliteit en het niet “goed doen” (hoofdstuk 2). Wanneer een soortgelijke diagnose wordt gesteld bij mensen heet het volgens Fokke en Sukke: diarree.

Door een rantsoen met meer celstof te voeren aan François langoeren wordt de mestkwaliteit beter. Dit is gebeurd door een broksoort te ontwikkelen die meer hemicellulose, cellulose en lignine bevat (hoofdstuk 5). Deze broksoort is echter minder smakelijk en wordt niet direct na het verstrekken, maar verspreid over de dag gegeten. Deze minder aantrekkelijke broksoort heeft geen effect gehad op het gedrag van de langoeren (hoofdstuk 6).

In hoofdstuk 7 wordt een proef beschreven uitgevoerd bij andere bladetende primaten (brillangoeren en colobusapen). Er is gekeken of er ook verbetering van de mestkwaliteit optreed wanneer ook zij een rantsoen kregen met meer en minder gemakkelijk verteerbare voedingsvezels.

De aan de langoeren in de westerse wereld gevoerde groentes bevatten veel water en weinig vezels en in verhouding veel gemakkelijk verteerbare koolhydraten. In een proef, beschreven in hoofdstuk 8, wordt weergegeven wat het effect is van het niet voeren van groentes aan dezelfde soort langoeren (mutslangoeren) die gehouden worden in de Apenheul en in Diergaarde Blijdorp in Rotterdam. Door het niet voeren van groentes verbetert de mestkwaliteit.

Uit de conclusies naar aanleiding van een proef uitgevoerd bij mutslangoeren (hoofdstuk 9) blijkt dat het voedsel langer in het verteringssysteem aanwezig is wanneer geen groentes worden gevoerd: de passagesnelheid is lager en de mestkwaliteit is beter. Hierbij is een rechtstreeks verband aangetoond tussen de hoeveelheid water en de hoeveelheid gemakkelijk verteerbare koolhydraten in de groentes.

Door middel van vetzuuranalyse van de rode bloedcellen en het serum van François langoeren, mutslangoeren en colobusapen na het verstrekken van een broksoort met soyaolie en met lijnzaadolie, is de conclusie getrokken dat de afbraak van het vet zowel in de magen van de langoeren plaatsvindt als in de dikke darm (hoofdstuk 10).

In hoofdstuk 11 heeft een vergelijking plaatsgevonden tussen alle gevoerde en geanalyseerde voeders aan de langoeren onder verschillende voeromstandigheden en de kwaliteit van de mest. Hieruit blijkt een rechtstreeks verband. De mest kwaliteit verbetert aanmerkelijk wanneer er meer vezels worden gevoerd en de rantsoenen minder water bevatten.

De conclusies van dit proefschrift hebben een belangrijke bijdrage geleverd tot een betere voersamenstelling voor bladetende apen in dierentuinen.

Dankwoord.

Artikelen lezen, een chaos op je bureau, correcties van medeauteurs waarvan je geen idee hebt hoe je die moet verwerken, proefdieren die ontsnapt of verplaatst zijn, behulpzame stagiaires en laboranten, wegbrengen van monsters, totaal onverwachte uitkomsten!

Waar doet een promovendus het voor en zeker een buitenpromovendus. En zoals Ronald Plasterk het reeds in 1996 zei (bedankt Koen Wink): ”Voor de meeste lezers bevat een proefschrift drie interessante pagina’s: het dankwoord, de samenvatting voor niet vakgenoten en het curriculum vitae. Dit zijn ook de enige drie hoofdstukken die de promovendus op eigen kracht heeft geschreven”

Marita Matthijsen schreef in september 2005 over “buitenpromovendi” in de NRC.: “De meesten zijn over de vijftig, een enkele wat ouder, een enkele wat jonger. Vroeger was elke promovendus een buitenpromovendus. Dat iemand door de overheid betaald zou worden om een proefschrift te schrijven was ondenkbaar. Maar deze mensen, die buitenpromovendi, die lang geleden afgestudeerd zijn, wat bezielt ze om naar iets te streven dat geen enkel ander voordeel oplevert dan een titeltje en meer prestige. Wat win je nou helemaal als je promoveert? Je mag je dr. noemen. Heeft een doctor in de maatschappij een hoger aanzien? Ik geloof niet dat een intellectuele titel nu nog tot verhoging van aanzien leidt. Lang voordat ze aan de rollator toe zijn, willen ze nog de archieven en bibliotheken in, om waar te maken wat ze ooit als droom onvoltooid hebben achtergelaten. Dat is wat mijn buitenpromovendi ook drijft: ze zoeken de jongen of het meisje van twintig in zichzelf terug, om af te maken wat ze ooit aan zichzelf beloofd hebben”.

Ronald Plasterk brengt de promovendus ook terug op aarde: “Het gebeurt nogal eens dat uit het dankwoord een weeë ons-kent-ons lucht opstijgt, die de vraag oproept waarom men privé-zaken niet privé afhandelt. Soms lees je houterige verklaringen aan het adres van de levensgezel, die je blij maken dat je er niet bij hoefde te zijn toen hij haar ten huwelijk vroeg”.

Toch zal ik, hopelijk in een stijl die niet lijkt op een Noord-Koreaanse lofzang op de president, een aantal personen bedanken die mij de afgelopen jaren geholpen hebben.

Anton Beynen, mijn promotor. Hij stelde mij de vraag toen ik als voedingsdeskundige van Diergaarde Blijdorp bij hem langs kwam met betrekking tot een onderzoekje van één van zijn studenten. “Je doet zoveel leuke onderzoeken, vind je het niet leuk om zelf te promoveren?”. En zo begon het: Anton; hartelijk dank voor al je opmerkingen, aanbevelingen, oplossingen, correcties etc etc.

Marcus Clauss: Lieber Freund und Kollege, herzlichen Dank fűr Deine Begutachtungen und fűr das Korrigieren. Es hat zu einen Grőssern Erfolg gefűhrt.

Ellen Dierenfeld: Dear friend: thank you very much. In 1994 the first steps for this promotion where made when I was 3 months in your office at the Wildlife Conservation Society in New York, were I compared the native food composition with diets fed to colobines in captivity. The whole digestion problems in colobines still keeps me engaged: the mechanism of digestion in colobines is still fascinating.

Dan natuurlijk de rest van mijn denktank, medeauteurs en illustrators: Wim Veen, Tjalling Huisman, John Cone, Celine Verheyen, Ellen Dierenfeld, Marcus Clauss, Carry Yeager, Elisabeth Bennet, William Bleisch, A Mitchell, Peter Klaver, Jan van der Kuilen, Robert Hovenier, Henk Everts, Hugo Wouterse, Ian Considine, Rob Doolaard en de vele stagieres.

De stagieres die geholpen hebben bij de diverse proeven welke uitgevoerd werden soms ‘s morgens vroeg, soms ’s avonds laat. Iedere proef had zijn eigen kenmerken met als hoogtepunt de opmerkingen van Kim van de Put. “Hé Joeke, één van de mutslangoeren is ontsnapt, wat nu?” en uiteindelijk nadat we de proef aangepast hadden “Hė Joeke, ik zie ineens die ontsnapte mutslangoer weer in het verblijf zitten, waarom hebben jullie dat niet even gezegd?” Ter informatie: de mutslangoer was zelf teruggegaan. Overigens zijn de gegevens van die proef niet verwerkt in deze promotie. Hartelijk dank Frauke Becher, Moniek Olsthoorn, Wendy Noordermeer, Marca Gresnight, Martine Frensdorf, Hans van Looy, Bart Verdoes en Kim van de Put. Zonder jullie was deze promotie echt niet gelukt!

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Ben Bothof en de vele anderen verzorgers van Blijdorp. Zonder jullie hulp hadden de proeven nooit uitgevoerd kunnen worden!

Wim van der Horst en Fernande Hazewinkels bedankt voor het kopiëren van de artikelen en voor het zetten van de puntjes op de i bij de verschillende teksten. Ook de medewerkers van Artis en Apenheul (Warner Jens, Hans van Weerd en Peter Klaver en hun verzorgers) hartelijk bedankt.

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Tot zover de welgemeende, zeker niet bagatelliserende, beroepsmatige bedankjes. Mocht ik iemand vergeten zijn: sorry. Mijn bedankjes zijn echt welgemeend!

Dan wil ik nu nog even de groeten doen aan mijn vrienden en familie en vooral aan Sjoerd en Femke: Bedankt dat jullie me de tijd gegund hebben om niet altijd met jullie te hoeven spelen en dat mamma Rieneke jullie daarom veelvuldig op sleeptouw heeft genomen. Rieneke, dank voor je stimulans. Dankzij jou was de stress, in elk geval voor mijn gevoel, hanteerbaar bij het schrijven van dit proefschrift….

Ik hoop dat de langoeren mede dankzij dit proefschrift nu en in de toekomst een beter rantsoen voorgeschoteld krijgen, want het zijn geweldig fascinerende apen!

In Fries:

Heit:” Heit, do fûnst it hiel aardich om Dr. Beiboer doedestiids te helpen mei syn promoasje. Gâns jierren letter is no dyn eigen soan doktor!

Sûnder dyn oanfiterjen om te studearjen en dyn earste lessen dierfersoargjen wie it nea safier kommen. Heit, tige tank! ”

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Levensloop

Joeke Nijboer is geboren op 7 juni 1955 te Siegerswoude een klein dorpje in Friesland. Na het opgroeien op de veeboerderij van zijn ouders haalde hij zijn HAVO diploma aan het Drachtster Lyceum te Drachten. Vervolgens ging hij studeren aan de toenmalige Bijzondere Hogere Landbouwschool in Leeuwarden alwaar hij in 1975 zijn diploma behaalde. Na stages op Nederlandse en Amerikaanse boerderijen begon bij hem het besef te rijzen dat zaken betreffende het milieu steeds belangrijker zouden worden. Het is dan ook niet verwonderlijk dat hij vervolgens een applicatiecursus milieukunde ging volgen aan het Van Hall Instituut in Groningen die hij afrondde in 1976.

In 1978 kreeg hij een betrekking bij Diergaarde Blijdorp te Rotterdam ter aanvulling van het kader. Hij zette daar het veterinair laboratorium op en was gedurende een groot aantal jaren hoofd van de voedselinkoop en bekwaamde zich daar ook in het formuleren van rantsoenen. In de loop van de jaren tachtig heeft hij zich onder andere ook bezig gehouden met het dierregistratiesysteen in Blijdorp en met het beheren van stamboeken voor bedreigde diersoorten. Sinds 1998 houdt hij zich full time bezig met het formuleren van rantsoenen en met uitvoeren van voedingsonderzoek.

Sinds eind jaren tachtig bezoekt hij regelmatig dierentuinvoedings-conferenties in Noord Amerika. Ook werden gedurende die jaren diverse studies gevolgd. Zo ontving hij in 1984 het Hoger Uniediploma Milieubeheer en in 1986 het Vakbekwaamheidsdiploma Plaagdierbestrijding. Sinds 1986 is hij coördinator plaagdierbestrijding in Diergaarde Blijdorp. Sinds 1998 is hij tevens hoofdredacteur van Pest Control News, het blad voor de ongediertebestrijding in Nederland en België.

Zijn voorliefde voor dierentuinvoedingsonderzoek werd nog meer gewekt na een stage gedurende 3 maanden op het Nutritional Department van de Wildlife Conservation Society in New York in 1994. Daar verzamelde hij onder leiding van Dr. Ellen Dierenfeld de geanalyseerde gegevens van het natuurlijk rantsoen van langoeren. Deze stage was ook de aanleiding tot zijn voorliefde voor langoeren en het schrijven van een proefschrift.

In 1999 nam hij het initiatief tot het coördineren van de Europese dierentuindierenvoeding door het organiseren van Europese voedingsconferenties beginnende in Rotterdam en het opzetten van de European Zoo Nutrition Research

Group (EZNRG). De voedingsconferenties worden om de twee jaar gehouden en op een wisselende locatie in een Europese dierentuin. In 2002 stond hij aan de basis van de verdere coördinatie van de Europese dierentuindierenvoeding. Hij is één van de oprichters van het European Zoo Nutrition Centre (EZNC). Hoewel hij zich in begin 2005 daaruit terugtrok houdt hij zich nu nog intensief bezig met het verbeteren van de voeding van dieren in Europese dierentuinen. Hij is dan ook actief betrokken bij de opvolger van EZNRG, de EAZA Nutrition Group.

Zoals eerder genoemd was 1994 een belangrijk jaar. Het bracht niet alleen het begin van zijn promotiearbeid maar ook leerde hij toen zijn huidige vrouw, Rieneke, kennen met wie hij inmiddels een zoon en dochter heeft (Sjoerd en Femke), twee fantastische kinderen!