Food for Thought: Lactating Coquerel's Sifaka (*Propithecus Coquereli*) Eat Foods High in Protein and Fiber During the Lean Season

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Abstract

Infant-bearing, Coquerel's sifaka (*Propithecus coquereli*) undergo gestation during a lean seasonal climate with weaning occurring during the abundant season. During this time, nutrient demand increases due to placental transport to the fetus and to the infant postpartum by milk. Females respond to this increased demand by ingesting larger food quantities, reducing expenditure, and/or using their nutrient stores. We collected foods (N=75) exploited by lactating females (N=10) in Ankarafantsika National Park, Madagascar to examine the nutritional landscape within which sifakas forage. We measured food nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), gross energy (GE) and ash to estimate crude protein (CP), available protein (AP), fiber, mineral content and metabolizable energy (ME). Two significant PCA (principal component analysis) axes corresponded to high protein and high fiber-low ME explaining 91.6% of the variance. Cluster 1 is categorized by foods that contained higher AP and cluster 2 is categorized by higher fiber foods. *P. coquereli* rely on a diverse range of foods inclusive of those with high AP and ME, but also high fiber foods with low ME. We hypothesize that the high fiber, low ME foods may be important for maintaining the gut microbiome.

Introduction

The nutrient content of foods eaten by wild primates is highly variable and resources are not interchangeable [1–3]. The nutrient quantities required for proper primate nutrition are contingent on body size, metabolism, digestive anatomy and physiology, sex, life history, and habitat quality [4–9]. Food selection indicates varying nutritional needs [10] by prioritizing nutrients to meet distinct nutritional goals within environmental constraints [11]. Assessing the nutrients and energy available from foods helps gauge these specific parameters within this contextual framework. One effective way to examine these constraints is to measure the nutrient content of foods consumed by individual animals to explore the nutritional options in their habitat.

The taxonomic Family Indriidae is composed of mostly folivorous-frugivorous primates endemic to Madagascar that have evolved an extensive small intestine and enlarged hindgut to assist with nutrient extraction [5]. The enlarged lower gut characteristic of hindgut fermenters consists of the caecum, a portion of the large intestine, and colon [12] that stretches to 13—15 times the animal's body length, thereby requiring a 24—48 hour gut-passage time in *Propithecus* spp. [13,14]. The lower gut serves as a fermentation chamber to aid in fiber digestion (Lambert 1998) with large populations of microbes housed in the caecum (Campbell et al. 1999). Microbes found in the caecum and colon are capable of fermenting fiber, in turn producing energy for indriids in the form of short-chain volatile fatty acids (primarily acetate, butyrate and propionate), as well as amino acids, vitamins and a host of other bioactive molecules that may benefit the host [15].

Indriids are challenged with the unpredictability in abundance and distribution of food resources due to the extreme seasonality within the region [16]. Additionally, the majority of lemur species including indriids give birth during the dry, lean season when resources are of lower quality (i.e., reduced protein and energy availability) and wean infants during the wet, abundant season when resources are higher quality (i.e., greater protein and energy availability) [17,18]. *P. coquereli* infants are born predominantly during the lean season from June—August and weaned during the abundant season from January—February [19,20]. This reproductive strategy intensifies the already high energetic demands on lactating females since infants are behaviorally and nutritionally dependent when resources are most seasonally depletive. As an example of a related species, female Verreaux's sifaka (*Propithecus verreauxi*) increase their overall food intake during late lactation; including increased intakes of crude protein, fat, non-structural carbohydrates and energy relative to males [6]. During gestation, sex differences in macronutrient intakes and energy were not present (Koch et al. 2017). Even with a greater nutrient intake during late lactation, lactating *P. verreauxi* lose 18% of their body weight throughout the dry season [21].

In the present study, we investigate the nutrient content of foods selected by lactating *P. coquereli* during the lean season. We assessed protein, fiber, energy, and minerals to explore the nutrients available to lactating females from which we characterize the nutritional landscape in which sifakas forage and feed.

Methods

Study Site

This study was conducted in Ankarafantsika National Park (ANP), Madagascar. ANP is a dry deciduous forest with a pronounced lean (dry) season from May to September [22] with the greater number of *P. coquereli* infants being born during this time; i.e., late May to

August [19,23]. Forested areas are experiencing anthropogenic disturbance from slash-and-burn agriculture, fire, human traffic, unregulated presence and herding of domestic cattle, bushmeat hunting and hole digging for *Dioscorea maciba* tuber extraction [24–26], which increases food scarcity during the lean season. Soils are either red, speckled, or white, with red soil containing the highest water content and white sand the lowest [25]. Many tree species grow in nutrient poor, acidic white sands and a thick layer of loose sand is present on the soil surface because of sandstone erosion [27,28]. Flora are speciose and the forest understory is moderately thick with sparse leaf litter (Lourenço & Goodman, 2006).

Plant Collection

The collection of plants that were consumed by ten habituated *P. coquereli* lactating females occurred from June to December of 2010 and 2011 for 93 hours over 52 weeks (26 consecutive weeks/season). Plant parts identified included: leaves, fruits, flowers, buds, and bark. Samples were stored in manila envelopes until they were transported to a propane drying oven at the end of each focal follow.

Plant Processing and Preservation

Samples were dried on-site in a propane oven at a maximum of 50°C using a max/min digital thermometer (HBE International Inc.) until a constant weight was reached for at least 48 hours [29]. Samples were weighed daily to determine dry weights and not exposed to direct sunlight to limit post-collection changes in nutrient composition. Samples were placed in 3M SCC Dri-Shield 2000 moisture barrier bags with silica gel and stored in plastic containers in a concrete storage area.

Scientific name identifications were confirmed by experts at Parc Botanique et Zoologique de Tsimbazaza, Antananarivo, Madagascar; Missouri Botanical Gardens, Antananarivo, Madagascar; Université d'Antananarivo – Faculté des Sciences; and ANP. Voucher herbarium specimens were sent to the Smithsonian National Zoological Park, Washington, D.C. and Missouri Botanical Garden, St. Louis, Mo. Permissions were granted to export plant material including names from the Direction Generale des Forets, Direction de la Valorisation des Ressources Naturelles, and Service de la Gestion Faune et Flore (N°128N_EV10/MG11).

Chemical Analyses and Calculations

Laboratory assays were conducted at the Nutrition Laboratory, Smithsonian National Zoo and Conservation Biology Institute. Dry food samples were re-dried at 55° C for a minimum of 48 hours and ground to achieve a homogeneous subsample. Plant material was ground using a Wiley mill or with a ceramic mortar and pestle depending on consistency and sample size and passed either through a 0.38 mm sieve (CHN procedure) or 0.86 mm sieve. Assays included: nitrogen (N) as an index for protein, neutral detergent fiber (NDF), acid detergent fiber (ADF), gross energy (GE) (kcal/g), and ash as an index for total mineral content. N content were measured using a combustion method (Dumas method) in a PerkinElmer 2400 Series II Analyzer (PerkinElmer, Waltham, MA). The ANKOM fiber procedure using an ANKOM Fiber 200 Analyzer or the Van Soest fiber procedure [30] were used for neutral detergent (NDF) and acid detergent fiber (ADF determination). We did not assay ADL (acid detergent lignin) which would have represented the indigestible fiber fraction and acknowledge this may have affected our results and interpretation. GE of samples (kcal/g) was measured using adiabatic bomb calorimetry to measure the heat from sample combustion. Pellets were formed from 0.25—0.75 g of sample and re-dried for one hour at 60°C. A Parr 1241 Adiabatic Calorimeter (Parr Instrument Company, Moline, IL) was used to measure GE. Samples were considered for re-assay if duplicates varied by >0.2 kcal/g. Total mineral content was determined by ashing the samples in a muffle furnace. Crucibles were filled with 0.25—0.50 g of sample and heated for six hours at 450° C.

We estimated crude protein (CP) following Maynard and Loosli [31]; available protein (AP) following [32] ; and metabolizable energy (ME) using values for energy not available from NDF from Campbell, et al. [33] and Conklin-Brittain, et al. [34].

$$CP = \%\ total\ protein = 6.25 * \%\mathrm{N}$$

$$AP = (CP - [ADF * 6.25 * N\{ADF\}])$$

$$ME = Protein\ ME + NPME$$

$$Protein\ ME = 4\frac{kcal}{g} * \ AP$$

$$Non-protein\ ME = GE - 5.86\frac{kcal}{g} * CP - 2.17\frac{kcal}{g} X\ NDF$$

The value of 2.17 kcal/g for energy not available from NDF was estimated using the value 61% NDF digestion factor [33] and accounting for the energy lost to microbial metabolism estimated by as 1kcal/g of NDF [34]. Thus, energy lost from NDF is estimated to be:

$$0.39 * 4\frac{kcal}{g}(undigested) + 0.61 * 1\frac{kcal}{g}(energy\ used\ in\ microbial\ metabolism) = 1.56\ kcal\ (undigested) + 0.61\frac{kcal}{g}(lost\ to\ microbial\ metabolism) = 2.17\frac{kcal}{g}$$

The mean value for NDF digestion was for captive foods[33], and thus likely represents a maximum for wild foods, so our estimated non-protein ME is likely an overestimate.

Statistical Analysis

A total of 139 plant samples were assayed, however, there were some duplicate samples of the same food type (e.g., fruit, leaf) and plant species collected from different locations or times. Duplicate samples were averaged to produce macronutrient values for a unique species-plant part except in the case of four species that displayed an apparent seasonal difference in nutrient composition (Table 1). These eight samples were treated as different foods, based on the macronutrient composition. This resulted in 75 unique sifaka foods for which we report data (Table 2). All nutrient results are reported on a dry matter basis to control for the effect of variable water content. Values are reported as mean ± SEM and range. Pearson's correlation was used to assess associations among nutrients assayed. Data were analyzed using SPSS 20.0, IBM Corp, Armonk NY.

Table 1
Foods consumed by *Propithecus coquereli* with seasonal differences

Botanical Name	**Malagasy Vernacular Name**	**Plant Part**	**Date**	**CP (%)**	**AP (%)**	**NDF (%)**	**ADF (%)**	**Ash (%)**
Abrahamia ditimena	DITIMENA	Leaves	July	7.9	5.2	45.0	36.6	4.8
Abrahamia ditimena	DITIMENA	Leaves	October	11.0	8.3	28.2	24.5	4.2
Seasonal change		July vs. October		39.2%	59.6%	-37.3%	-33.1%	-12.5%
Dalbergia bracteolata	VAHAFISAKA	Leaves	July	13.4	12.0	33.0	20.2	5.2
Dalbergia bracteolata	VAHAFISAKA	Leaves	October	19.1	17.7	21.6	13.7	4.2
Seasonal change		July vs. October		42.5%	47.5%	-34.6%	-32.2%	-19.2%
Dalbergia trichophylla	MANARY	Fruit	September	10.4	7.8	40.3	31.2	3.5
Dalbergia trichophylla	MANARY	Fruit	November	18.1	15.0	43.7	32.8	3.0
Seasonal change		September vs. November		74.0%	92.3%	8.4%	5.1%	-14.3%
Grangeria porosa	MAEVALAFIKA	Leaves	June	12.0	10.4	42.1	26.6	3.9
Grangeria porosa	MAEVALAFIKA	Leaves	October	7.6	6.4	54.5	34.3	3.4
Seasonal change		June vs. October		-36.7%	-38.5%	29.5%	29.0%	-12.8%

Table 2
Plants selected as food resources by lactating *Propithecus coquereli* during Madagascar's lean (dry) season, including their respective nutrient and energy values

+Botanical Name (genus + specific epithet)	Botanical Family	+Malagasy Vernacular Name	Plant Part	CP (%)	AP (%)	NDF (%)	ADF (%)	Ash (%)	ME (kcal/g)
Abrahamia ditimena	ANACARDIACEAE	DITIMENA	Leaves	11.0	8.3	28.2	24.5	4.2	3.8
Abrahamia ditimena	ANACARDIACEAE	DITIMENA	Leaves	7.9	5.2	45.0	36.6	4.8	3.4
Abrahamia ditimena	ANACARDIACEAE	DITIMENA	Bark	2.2	0.0	79.2	75.2	3.4	2.8
Abrahamia ditimena	ANACARDIACEAE	DITIMENA	Fruit	4.5	n/a	14.8	8.2	2.5	n/a
Abrahamia spp.	ANACARDIACEAE	MANGA	Fruit	3.5	3.4	8.6	6.2	1.9	3.6
Abrahamia spp.	ANACARDIACEAE	MANGA	Leaves	9.9	8.4	50.4	39.8	4.8	n/a
*Albizia boivinii** or Unidentified	FABACEAE	KITSAKITSANALA	Leaves	20.3	18.7	26.1	17.7	4.9	3.5
Albizia mainaea	FABACEAE	ALIBIZAHA	Leaves	16.9	15.8	40.2	15.0	4.6	3.8
Astrotricha spp.	MELIACEAE	VALOMAMAY	Fruit	8.6	7.7	19.3	14.6	5.2	5.0
Bathiorhamnus spp.	RHAMNACEAE	KABIJALAHY	Leaves	13.0	10.8	46.2	33.05	3.2	3.8
Bussea perrieri	FABACEAE	MIMOZA	Leaves	23.7	22.1	23.0	15.2	6.0	3.7
*Capurodendron perrieri** or *Asteropeia amblyocarpa** or *Securinega* spp.*	SAPOTACEAE or ASTEROPEIACEAE or PHYLLANTHACEAE	HAZONJIA	Leaves	10.4	8.4	45.9	34.7	5.8	3.6
Combretum spp.	COMBRETACEAE	MANAKOBONGO	Fruit	7.3	6.5	45.1	35.2	3.5	3.3
Commiphora spp.	BURSERACEAE	MATAMBELONA	Leaves	13.6	11.8	17.1	14.5	5.6	3.6
Commiphora spp.	BURSERACEAE	MATAMBELONA	Buds	7.8	7.0	24.2	19.2	4.2	n/a
Crateva excelsa	CAPPARIDACEAE	PAMBA	Flowers	15.1	13.4	26.8	17.3	6.9	3.3
Cynanchum spp.	ASCLEPIADACEAE	RAHAMATSATSO	Flowers	6.1	5.6	42.9	38.4	n/a	3.0
Dalbergia bracteolata	FABACEAE	VAHAFISAKA	Leaves	13.4	12.0	33.0	20.2	5.2	3.0

AP = available protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NPGE = non-protein gross energy; ME = metabolizable energy; ash = total minerals.

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Dalbergia bracteolata	FABACEAE	VAHAFISAKA	Leaves	19.1	17.7	21.6	13.7	4.2	3.8
Dalbergia trichophylla	FABACEAE	MANARY	Leaves	18.3	17.4	20.2	14.9	4.7	3.7
Dalbergia trichophylla	FABACEAE	MANARY	Fruit	18.1	15.0	43.7	32.8	3.0	4.5
Dalbergia trichophylla	FABACEAE	MANARY	Fruit	10.4	7.8	40.3	31.2	3.5	4.8
Dalbergia trichophylla	FABACEAE	MANARY	Flowers	16.1	13.07	n/a	30.0	n/a	n/a
Dichapetalum spp.	DICHAPETALACEAE	FANTSIKATRA	Flowers	12.8	11.7	n/a	30.1	3.4	n/a
Diospyros spp.* or *Diospyros tropophylla** or *Casearia nigrescens**	EBENACEAE or SALICACEAE	HAZOMAFANA	Leaves	17.1	16.3	27.3	21.5	3.8	4.0
Entada spp.	FABACEAE	ROIMENA	Flowers	16.9	15.2	34.8	22.7	n/a	n/a
Entada spp.	FABACEAE	ROIMENA	Fruit	23.9	22.0	18.4	18.3	n/a	n/a
Eucalyptus spp.* or *Eucalyptus camaldulensis**	MYRTACEAE	KININIMA	Leaves & bark	3.0	0.0	81.8	72.7	2.7	2.6
*Gambeya boiviniana**	SAPOTACEAE	VOATSIKIDY	Leaves	22.2	20.9	44.8	28.3	7.2	3.3
Garcinia verrucosa	CLUSIACEAE	NATOVAVY	Leaves	8.5	6.4	40.6	33.7	4.0	3.8
Garcinia verrucosa	CLUSIACEAE	NATOVAVY	Fruit	6.2	5.7	17.7	12.0	3.6	4.0
Garcinia verrucosa	CLUSIACEAE	NATOVAVY	Leaf buds	9.4	8.4	31.7	18.8	4.4	4.4
Grangeria porosa	ROSACEAE	MAEVALAFIKA	Bark	5.9	1.4	79.6	68.5	19.4	1.9
Grangeria porosa	ROSACEAE	MAEVALAFIKA	Leaves	7.6	6.4	54.5	34.3	3.4	2.9
Grangeria porosa	ROSACEAE	MAEVALAFIKA	Leaves	12.0	10.4	42.1	26.6	3.9	3.5

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Grangeria porosa	ROSACEAE	MAEVALAFIKA	Leaf buds	9.0	7.3	37.2	25.2	9.1	3.5
Grangeria porosa	ROSACEAE	MAEVALAFIKA	Fruit	8.6	6.9	50.6	34.3	3.7	3.5
Grewia ambongensis	TILIACEAE	SELIVATO	Fruit	18.2	17.0	29.1	22.7	6.5	4.2
Grewia ambongensis	TILIACEAE	SELIVATO	Leaves	22.2	20.8	25.4	15.8	7.8	3.2
Grewia spp.	MALVACEAE	SELIALA	Fruit	5.5	3.5	74.4	57.7	3.3	2.7
Landolphia gummifera	APOCYNACEAE	PIRA	Fruit	3.8	3.2	51.6	34.5	2.1	2.9
Macphersonia gracilis	SAPINDACEAE	MAROAMPOTOTRA	Fruit	4.9	3.3	61.5	38.6	3.3	2.6
Malleastrum gracile	MELIACEAE	ANDRIAMANAMORA	Leaves	19.3	17.6	50.2	38.5	5.2	2.8
Mammea punctata	CLUSIACEAE	TSIMATIMANOTA	Leaves	8.2	6.6	49.1	38.4	3.5	3.7
Mammea punctata	CLUSIACEAE	TSIMATIMANOTA	Fruit	3.4	2.9	22.4	9.9	2.3	4.0
Mascarenhasia spp.*	APOCYNACEAE	GODROA	Leaves	13.8	12.6	24.2	18.6	5.9	3.8
Mimusops spp.	SAPOTACEAE	HAZOPIKA	Fruit	2.9	2.1	64.2	41.0	2.2	3.0
Monanthotaxis spp.	ANNONACEAE	FOTSIAVADIKA	Leaves	16.5	15.0	28.3	19.3	3.9	n/a
Monanthotaxis spp.	ANNONACEAE	FOTSIAVADIKA	Buds	15.6	13.7	36.2	23.7	n/a	3.6
Noronhia spp.	OLEACEAE	HAZOTSIFAKA	Leaves	12.5	11.0	35.5	27.6	5.3	3.5
Noronhia spp.	OLEACEAE	HAZOTSIFAKA	Bark	8.9	0.0	71.2	62.7	11.2	2.1
Ochna ciliata	OCHNACEAE	MORAMENA	Leaves	17.4	14.6	32.5	22.9	3.9	3.6
*Omphalea oppositifolia**	EUPHORBIACEAE	VOASALAY	Flowers	11.0	10.1	46.2	36.7	3.9	3.4
*Passiflora foetida**	PASSIFLORACEAE	BONGAPISO	Leaf buds	12.5	11.1	44.1	22.8	8.1	3.0
*Passiflora foetida**	PASSIFLORACEAE	BONGAPISO	Leaves	27.1	24.4	37.9	26.7	6.8	3.1
*Passiflora foetida**	PASSIFLORACEAE	BONGAPISO	Fruit	14.3	13.2	35.8	15.3	5.4	3.1

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Polyalthia spp.	ANNONACEAE	AMBALAHY	Leaves	14.8	11.9	48.1	28.2	4.2	3.4
Polyalthia spp.	ANNONACEAE	AMBALAHY	Flowers	19.7	19.1	22.4	25.5	4.7	4.1
Polycardia libera	CELASTRACEAE	MAMOARAVINA	Leaves	12.6	12.2	20.8	16.2	6.3	4.2
Polycardia libera	CELASTRACEAE	MAMOARAVINA	Flowers	5.3	5.0	69.4	16.9	n/a	n/a
*Poupartia sylvatica** or *Poupartia* spp.* or *Sclerocarya birrea**	ANACARDIACEAE	SAKOALA	Leaves	13.2	11.0	19.1	15.8	3.4	3.9
*Poupartia sylvatica** or *Poupartia* spp.* or *Sclerocarya birrea**	ANACARDIACEAE	SAKOALA	Flowers	7.9	6.8	24.9	21.4	2.9	3.9
Rhopalocarpus similis	RHOPALOCARPACEAE	HAZONDRINGITRA	Fruit	5.5	4.8	30.8	17.1	2.6	3.4
Sorindeia madagascariensis	ANACARDIACEAE	VOATSIRINDRANA	Fruit	4.9	4.2	16.8	10.2	3.1	3.6
Sorindeia madagascariensis	ANACARDIACEAE	VOATSIRINDRANA	Flowers	9.6	9.4	8.3	6.5	3.4	4.2
Strychnos madagascariensis	LOGANIACEAE	VAKAKOA	Leaf buds & leaves	19.6	17.6	19.8	12.8	4.8	4.0
Strychnos madagascariensis	LOGANIACEAE	VAKAKOA	Leaves	9.0	7.5	33.1	23.3	4.3	3.7
Tabernaemontana coffeoides or *Mimusops* spp.	APOCYNACEAE or SAPOTACEAE	HAZOPIKA	Leaves	11.5	10.1	27.2	21.6	4.8	3.9
Tectonia grandis	VERBENACEAE	KESIKA	Fruit	6.2	4.7	78.5	64.6	4.1	2.7
Terminalia boivinii	COMBRETACEAE	AMANINOMBY	Leaves	8.2	7.5	52.9	43.7	3.3	2.9

AP = available protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; NPGE = non-protein gross energy; ME = metabolizable energy; ash = total minerals.

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Treculia perrieri	MORACEAE	TSITIPAHA	Fruit	13.2	10.3	30.1	25.1	10.0	3.4
Trilepisium madagascariense	MORACEAE	KILILO	Leaves	11.1	10.3	27.9	19.0	6.5	3.2
Unidentified	UNIDENTIFIED	UNKNOWN FALLEN TREE	Bark	2.5	0.6	81.2	65.1	n/a	2.4
Unidentified	UNIDENTIFIED LIANA	UNKNOWN LIANA	Leaves	20.5	19.5	22.2	12.1	7.2	3.9
Unidentified	UNIDENTIFIED LIANA	UNKNOWN LIANA	Leaves	16.6	15.5	30.8	25.7	10.0	3.7

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published sources.

*Annotated botanical names were initially unidentified specimens associated only with Malagasy vernacular names. Consultations with Missouri Botanical Garden - Madagascar and Université d'Antananarivo - Faculté des Sciences resulted in translated suggestions of possible species endemic to the Ankarafantsika National Park region and within the distribution range of *P. coquereli* and are not based on taxonomic identification of actual plant specimens.

Exploratory statistics were used to describe the variation in sifaka foods. Principal component analysis (PCA) was conducted on the nutrient values to reduce the number of parameters (CP, AP, NDF, ADF, GE, ME, and ash). Only axes with an eigen-value greater than one were considered significant. The PCA was considered significant if Bartlett's Test for Sphericity was significant and the Kaiser-Meyer-Olkin measure of sampling adequacy was greater or equal to 0.5 [35]. The number of significant axes from the PCA was used to set the *k* value for the *k*-means cluster analysis on the same parameter set.

Results

Nutrient values for the 75 unique plant foods are given in Table 2. The sifakas selected foods representing 48 unique plant taxa with a wide range of nutrient content. AP, digestible protein not bound in fiber, ranged from 0.0–24.4%, with a mean of 10.3 ± 0.7% and median of 10.2%. NDF ranged from 8.3–81.8% with a mean of 38.2 ± 2.1% and median of 34.8%. ADF ranged from 6.2–75.2% with a mean of 27.8 ± 1.8% and median of 23.7%. ME ranged from 1.92 kcal/g to 4.96 kcal/g, with a mean of 3.49 ± 0.07 kcal/g and median of 3.56 kcal/g. Ash (total minerals) ranged from 1.85–19.37%, with a mean of 4.95 ± 0.32% and median of 4.22%. Four foods showed seasonal differences in nutrient composition, with the highest percentage of change in the amount of protein in manary (*Dalbergia trichophylla*) fruit from the end of the lean to the beginning of the wet season (Table 1).

Except for bark, plant part does not categorize sifaka foods by nutrient composition, as all plant part categories had examples of high and low values for all nutrients. For example, the mean and range of NDF content of leaves (35.8%, 17.1 – 81.8%) was virtually the same as the mean and range of NDF for fruit (37.7%, 8.6 – 78.5%). Although the mean value for available protein for leaves (12.7±0.9%) was numerically higher than that for fruit (7.6±1.3%), the range again was essentially identical for the two plant parts (0 – 24.4% and 0 – 22.0%). Bark contained mostly fiber, with essentially no available protein (Table 2).

The best fit PCA model contained only five of the seven parameters (CP, AP, NDF, ADF, and ME). The best model found two significant axes (eigen-values greater than one) that can be categorized as high protein and high fiber. These two axes (protein factor and fiber factor) explained 91.6% of the variation in nutrient content between the foods. Bartlett's Test of Sphericity was significant (Chi-square = 435. 6, df = 10, p<0.001) and the Kaiser-Meyer-Olkin measure of sampling adequacy was 0.659, suggesting that sampling is adequate.

Estimated ME was significantly negatively correlated with the fiber factor score from the PCA (r = -0.867, p<0.001; Figure 1) but was not associated with the protein factor score. Ash was positively correlated with the protein factor score (r = 0.314, p = 0.012) but was not correlated with the fiber factor score.

The cluster analysis had *k* set to 2 based on the number of significant axes from the PCA. Cluster 1 foods (N=52) were higher in AP and lower in fiber (Table 3). The foods in cluster 2 (N=14) were higher in fiber and lower in estimated ME (Table 3). Nine foods could not be ascribed to a cluster because they were missing GE data, and thus an estimated ME could not be calculated. Figures 2 through 4 display how the foods in the two clusters differ. Cluster 1 foods displayed a positive correlation between the protein and fiber factor scores ($r = 0.580$, $p<0.001$, Figure 2) while cluster 2 foods showed no association ($r = -0.136$, $p=0.642$, Figure 2). Both cluster 1 and cluster 2 foods had negative correlations between estimated ME and the fiber factor score ($r = -0.728$, $p<0.001$ and $r = -0.855$, $p<0.001$). Cluster 1 foods had a tendency for estimated ME to be negatively associated with the protein factor score ($r = -0.270$, $p=0.053$), but there was no association between estimated ME and the protein factor score for cluster 2 foods.

Table 3
Nutrients in wild plant foods consumed by *P. coquereli* compared to components of captive lemur diet supplements

Cluster Number	**Analysis Parameter**	**CP (%)**	**AP (%)**	**NDF (%)**	**ADF (%)**	**Ash (%)**	**GE (kcal/g)**	**ME (kcal/g)**
1: High AP/ Low Fiber	Mean	13.4	12.0	31.1	22.0	5.0	4.7	3.6
	N	52	52	52	52	50	52	52
	Std. Error of Mean	0.76	0.73	1.47	1.17	0.26	0.06	0.06
	Median	13.1	11.1	30.5	21.5	4.8	4.6	3.6
2: High Fiber/ Low ME	Mean	5.6	3.3	66.4	52.7	5.0	4.4	2.5
	N	14	14	14	14	13	14	14
	Std. Error of Mean	0.65	0.74	3.43	4.07	1.35	0.08	0.13
	Median	5.7	3.3	67.7	50.9	3.4	4.4	2.5
Total	Mean	11.8	10.1	38.6	28.5	5.0	4.6	3.4
	N	66	66	66	66	63	66	66
	Std. Error of Mean	0.73	0.74	2.25	1.99	0.34	0.05	0.08
	Median	11.0	10.1	35.6	24.8	4.3	4.6	3.4
Marion[+]	Guaranteed Analysis	≥ 23%		≥ 21%	13% -16%	≤ 7%		
Mazuri[*]	Guaranteed Analysis[‡]	≥ 23%			≤ 14%[‡]	≤ 9%		

CP= crude protein; AP = available protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ash = total minerals; GE = gross energy; ME = metabolizable energy.

[+] Marion Zoological Inc., Plymouth, Minnesota, USA. SKU# LEL B25, Leaf Eater Foods Biscuit

[*] Mazuri Exotic Animal Nutrition, St. Louis, Missouri, USA. SKU# 0001472 and 0001448, Leaf-Eater Primate Diet, Biscuit and Mini-Biscuit, respectively.

[‡] Note: Mazuri's guaranteed analysis for fiber is measured as Crude Fiber (CF). NDF and ADF values are not publicly available.

Figure 3 displays how cluster 1 foods are lower in ADF (though there is overlap) and both higher and more variable in estimated ME. Figure 4 displays the lower and less variable AP for cluster 2 foods. In addition, there is no relationship between AP and ADF for cluster 1 foods (Figure 4), but a significant decline in AP with ADF for cluster 2 foods ($r = -0.717$, $p=0.004$). The ratio of AP to CP differed between clusters 1 (0.88±.01) and 2 (0.53±0.1; $p<0.001$), indicating that a greater percentage of protein was bound to the ADF fraction for cluster 2 foods. Cluster 2 foods had a lower protein-to-fiber ratio whether expressed as CP-to-NDF (0.48±0.04 versus 0.09±0.01, $p<0.001$) or CP-to-ADF (0.70±0.05 versus 0.12±0.02, $p<0.001$).

Cluster 2 foods were comprised of all 4 bark samples, 6 of 18 fruit samples, 4 of 29 leaf samples, but no buds or flowers. There were 6 samples, 3 from cluster 1 and 3 from cluster 2, that overlap in the fiber and protein factor space (Figure 2). The cluster 1 foods were lower in NDF (44.3±0.7% versus 50.4±0.7%, p=0.004) with no overlap, but otherwise did not differ from the cluster 2 foods (Table 4).

Table 4
Foods from clusters 1 and 2 overlapping in the protein factor-fiber factor space+

Botanical Name	**Plant Part**	**CP (%)**	**AP (%)**	**NDF (%)**	**ADF (%)**	**Ash (%)**	**ME (kcal/g)**
Abrahamia ditimena	Leaves	7.9	5.2	45.0	36.6	4.8	3.4
Combretum spp.	Fruit	7.3	6.5	45.1	35.2	3.5	3.3
Cynanchum spp.	Flowers	6.1	5.6	42.9	38.4	n/a	3.0
Mean Cluster 1*		7.1 ±0.54	5.8 ±0.38	44.3 ±0.71	36.7 ±0.94	4.2 ±0.69	3.2 ±0.12
Grangeria porosa	Fruit	8.6	6.9	50.6	40.7	3.7	3.5
Landolphia gummifera	Fruit	3.8	3.2	51.6	34.5	2.1	2.9
Mammea punctata	Leaves	8.2	6.6	49.1	38.4	3.5	3.7
Mean Cluster 2*		6.8 ±1.54	5.6 ±1.18	50.4 ±0.74	37.9 ±1.78	3.5 ±0.48	3.4 ±0.22
+See Figure 2.							
*Foods differed by cluster in neutral detergent fiber (NDF) (F=35.769, df=1, p=0.004).							

Discussion

We found that lactating *P. coquereli* exploited a nutritionally diverse set of foods that varied widely for all measured nutrients and included many high fiber foods. The PCA indicated that available protein, fiber and metabolizable energy accounted for over 91% of the variation among these foods. Our analysis revealed two potential categories of foods in our dataset, visually represented in Figure 2. The relationship between ME and ADF (Figure 3) and AP and ADF (Figure 4) visually demonstrates the separation between the clusters for fiber. However, estimated ME and AP shows considerable overlap between the two clusters, suggesting that sifaka foods could be described by a nutritional gradient. This approach is supported since some foods were moderate to higher in protein and metabolizable energy while lower in fiber, and other foods were lower in protein and metabolizable energy while higher in fiber. The gradient approach may better reflect the continuous nature of nutrient values, particularly for foods on the cluster boundaries (Figures 1, 2, and 4). However, the six foods overlapping in protein and fiber factor space do differ in NDF (Table 4) and the two clusters vary in the proportion of fiber bound to ADF. Both these factors support the hypothesis that these foods cluster into at least two nutritionally distinct groups. We propose that these two food types will have different physiological and metabolic effects, with cluster 1 foods contributing more to the ingesting sifaka's nutritional status directly while cluster 2 foods will affect nutritional status through effects on the sifaka gut microbiome.

Protein and fiber were the most consistently variable nutrients in the sifaka foods, which also varied considerably in the protein-to-fiber ratio. Primates are estimated to require a minimum of 14% protein per dry matter basis for reproduction, 7—11% for growth and development [36], and 6.4—8% crude protein in their diet to satisfy maintenance nutritional requirements [37]. The cluster 1 foods consumed by lactating *P. coquereli* had a mean of 12.0% available protein, which exceeds minimum protein requirements for primate maintenance, and growth and development, while nearly meeting the estimated reproductive nutritional requirements. Cluster 1 foods had a high ratio of AP-to-CP, supporting the hypothesis that they are good protein sources.

Lactating *P. coquereli* appear to have a diet quite high in fiber (means of 38.2% NDF, 27.8% ADF) with a relatively low protein-to-fiber ratio without experiencing adverse effects and routinely consumed high fiber foods during the lean season (Table 3). Frequently consumed foods of gestating ring-tailed lemurs (*Lemur catta*) during the dry season contained less than 21% ADF [3]. During lactation, eight of ten of the most frequently consumed foods contained less than 30% ADF, while none of the foods contained over 50% ADF [3]. The black-and-white ruffed lemur (*Varecia variegata*), consumed fruits, leaves and flowers with ADF content of approximately 30% [38]. The average ADF content of leaves eaten by the larger-bodied Indri (*Indri indri*) was 53%, and the fruit, leaves and flowers consumed by diademed sifakas (*Propithecus diadema*) averaged between 30 and 50% ADF [39]. The fiber levels for these larger lemur species are comparable to our results for *P. coquereli*. Although, the sifakas did include many high protein/low fiber foods in their diet, suggesting that exploiting different foods has functionally distinctive physiological and metabolic consequences.

High fiber food consumption may be a residual effect of lactating *P. coquereli* unselectively exploiting the foods available in the forest during the lean season. We emphasize that this also has biological relevance, since it provides an assessment of seasonally available nutrients consumed during the critical period of infant development. During the lean season in a dry deciduous forest the availability of foods high in available protein and metabolizable energy may be insufficient, thereby constraining females to select difficult to digest resources to meet energy requirements. Perhaps the increased demand placed by lactation in conjunction with the food constraints of the lean season force sifakas living in dry deciduous forest to ingest the high fiber foods.

However, *Propithecus* spp. are hindgut fermenters [5] with highly specialized gut microbiomes that vary depending on seasonal fruit availability [40]. Dietary plant fiber only become nutritious after its microbial conversion into vital nutrients like short-chain fatty acids [41], facilitated by specific cellulose-degrading microbes present in the sifaka gut and an increased functional capacity for fiber metabolism [42]. The specialized morphology of hindgut fermenters (enlarged caecum and elongated colon) could enable the efficient digestion of fibrous materials, increasing nutrient extraction from difficult to digest resources. Our findings are consistent with previous studies that have shown sifakas to be seasonally flexible folivores, a novel dietary strategy that may mitigate potential energetic deficits [43–45]. Recent evidence demonstrates sifakas possess molecular adaptions to folivory including rapidly evolving gene pathways that aid in xenobiotic metabolism and nutrient absorption, which may assist in the detoxification of plant compounds while maximizing nutritional gain from leaves [46]. This capacity for augmented nutrient uptake [46] would be advantageous to foraging throughout periods of pronounced seasonality in Madagascar [18,47].

Variation in dietary fiber is a critical component to understanding gut microbiomes in folivores and has been shown to affect microbial diversity in *P. coquereli* [42]. Sifaka gut microbiomes have been found to be significantly richer and more diverse in comparison to generalist and frugivorous lemurs [42]. Less inter-individual variation in sifaka gut microbiomes is exhibited relative to frugivorous *V. variegata* and generalist *L. catta*, suggesting that sifakas may be less flexible in terms of their diet [42] and more susceptible to habitat disturbance [48].

Captive *P. coquereli* provisioned with a more diverse diet that included local wild plant species had significantly richer, more diverse gut microbiomes in comparison to when their standard diet was supplemented with winged-sumac only [48]. Significantly higher concentrations of short-chain fatty acids, including acetate and propionate, and moderately greater concentrations of butyrate were present in *P. coquereli* colonic metabolomes when provisioned a more diverse diet [48]. Additionally, the same study found that individuals given the opportunity to forage more naturally in forested enclosures, even for limited durations, maintained greater gut microbiome diversity relative to conspecifics without forest access (Green et al., 2018). This supports that fiber consumption can have a profound influence on gut microbiome structure and function. It is possible that a high-fiber diet is a requirement for sifakas to maintain their coevolved microbiota. We posit that many if not all the cluster 2 foods in our study may have a greater effect on the sifaka gut microbiome than a direct nutritional effect on the host animal. In other words, cluster 2 foods may be important for maintaining gut health by feeding the microbiome, while cluster 1 foods more directly affect the nutritional plane of the sifakas.

Sifakas are exceptionally difficult to maintain in captivity due to their specialized digestive anatomy and highly folivorous diet [49,50]. Our results suggest that incorporating high-fiber foods (ADF greater than 30% or even 40%) into captive diets would better replicate foods consumed in the wild. Table 3 highlights two leading commercial products for leaf-eating primates in various life cycle stages, health, and seasonality versus our field data collected on lactating sifakas during the lean season. The commercial supplements contain higher concentrations of protein (CP) and lower concentrations of fiber. Both Marion and Mazuri provide their products as supplements to foraging and non-foraging fruit and vegetable produce diets. Because of this, percent nutritional values of the various nutrients do not represent the overall lemurs' diet, but only that of the commercial product itself. Similarly, food selection in the wild depends on environmental factors and does not necessarily reflect the ideal composition for the health of sifakas without food supply constraints as in captivity. While we acknowledge the limitations of juxtaposing a partial wild diet to a partial captive diet, it is presented here to highlight the importance of incorporating nutritional diversity in captive diet design based on wild plant foods acquired by lemurs. We suggest that incorporating foods like the cluster 2 foods in this study may be helpful for dietary management of captive sifakas, possibly by improving gut health through effects on the microbiome.

Consistent with previous studies [11,29,51–53], our results confirm that botanical category (e.g., fruit versus leaf) is a poor means by which to assess the nutritional contribution a food will make to animals that consume it. Fruit is often equated with high water and high non-structural carbohydrate (sugar) content; however, wild fruits can be substantially different in nutrient profile from domesticated fruits, and often are similar to leaves, buds, and flowers, as seen in our study. The fruits in this study were not different from leaves in fiber content. The NDF content of fruit in our study ranged from 8.6–78.5% and the mean NDF for fruit (37.7%) was numerically higher than the mean NDF for leaves (35.8%). Sifakas ingest high fiber foods, whether those foods are classified as leaves, fruit, flowers, or buds. Our results also confirm that wild plant foods can vary seasonally in nutrient content, cautioning that the nutritional consequences of consuming some foods can differ by time of year.

In summary, infant-bearing *P. coquereli's* employ a mixed-diet strategy consuming foods with wide ranges in percent nutrient content to compensate for nutrient deficiencies in multiple plant parts and food availability. Food sources clustered into two categories: high in protein and low-to-moderate in fiber; or high in fiber and low in metabolizable energy.

Declarations

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Author Contributions

ACR designed the project and collected the data. ACR and MLP assayed the samples. MLP conducted statistical analyses. ACR and MLP co-wrote the manuscript.

Statement of Ethics

This research complied with protocols approved by the University of Toronto Animal Care Committee (Protocol #: 2000), adhered to the legal requirements of Madagascar and followed the American Society of Primatologists' Principles for the Ethical Treatment of Primates. Research permits were issued in Madagascar by the Ministry of Environment, Water and Forests (Permit #: N°239/11/MEF/SG/DGF/DCB SAP/SCB). Export permits were issued in Madagascar by the Director of Natural Resources. Imports were approved by the United States Department of Agriculture and U.S. Fish and Wildlife Service.

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Data Availability Statement

The data presented here are represented by Table 1. The full data set is available in ACR's dissertation.

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Figures

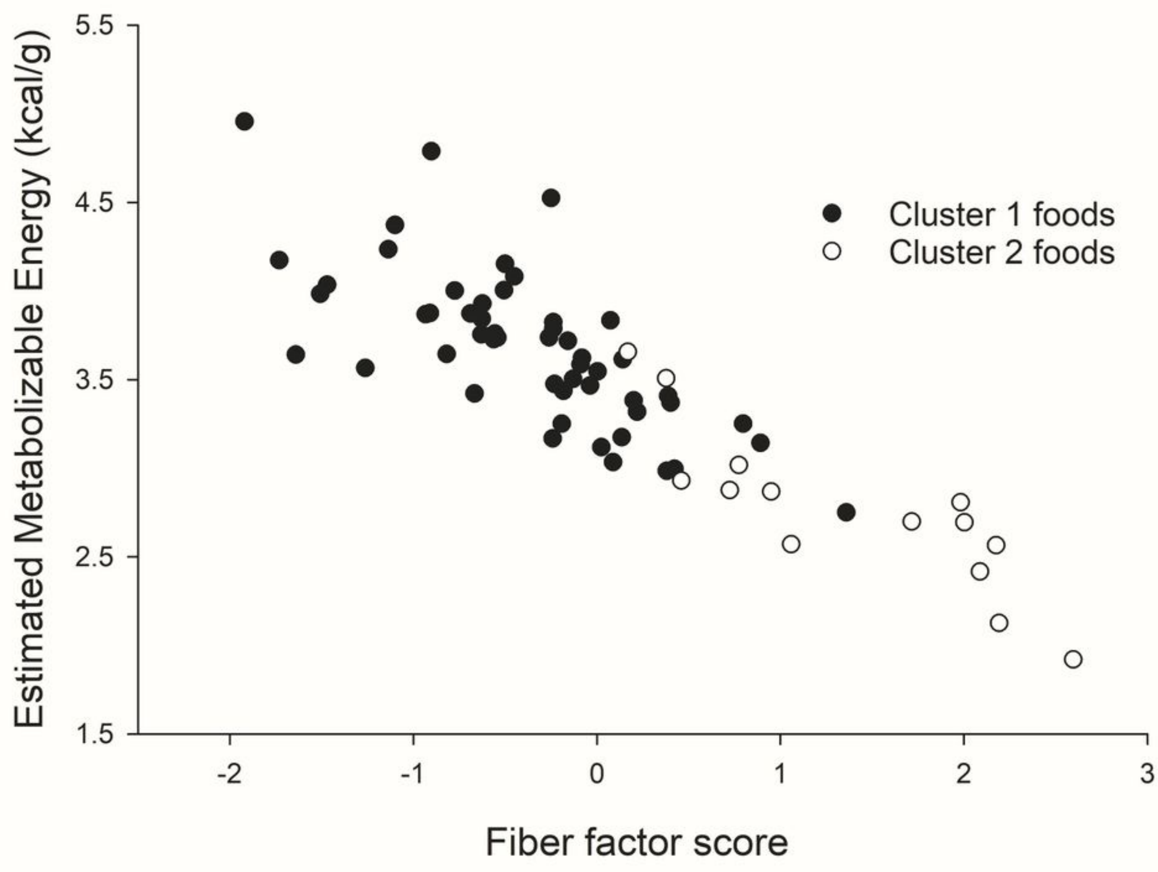

Figure 1

Relationship between metabolizable energy (ME) and fiber by cluster in foods consumed by lactating *P. coquereli*

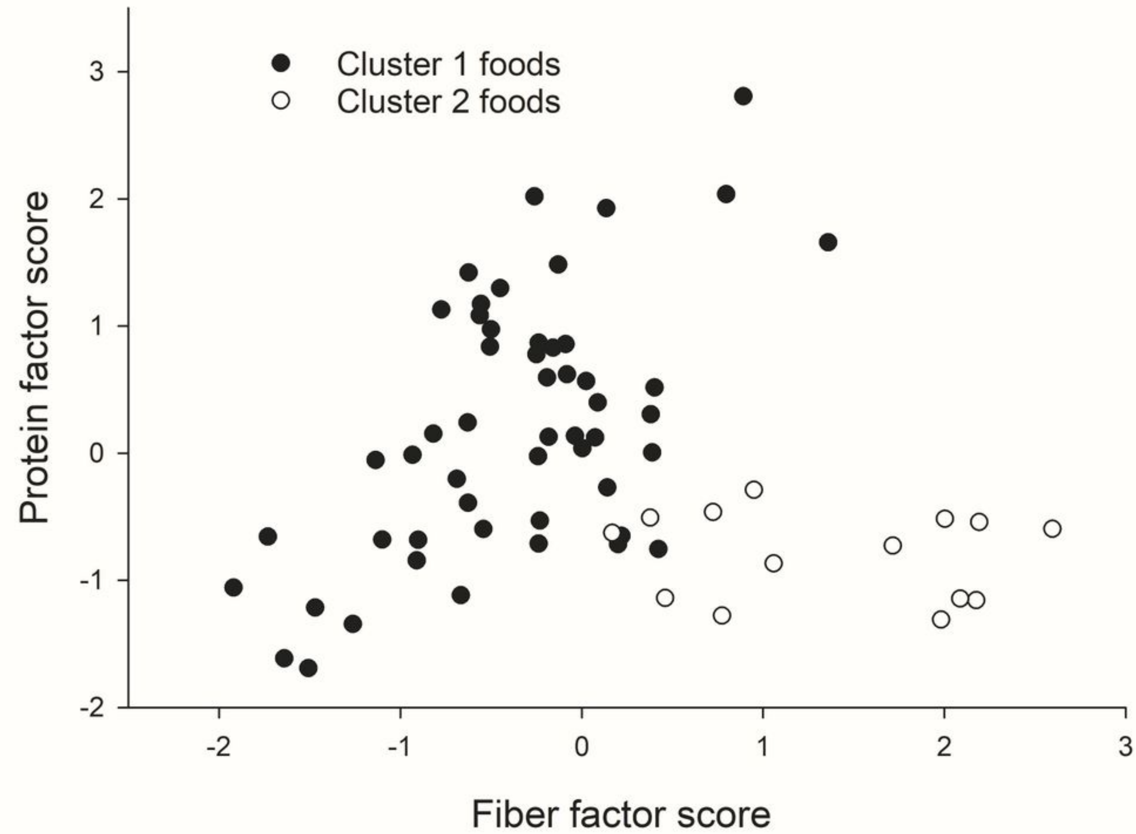

Figure 2

Relationship between protein and fiber by cluster in foods consumed by lactating *P. coquereli*

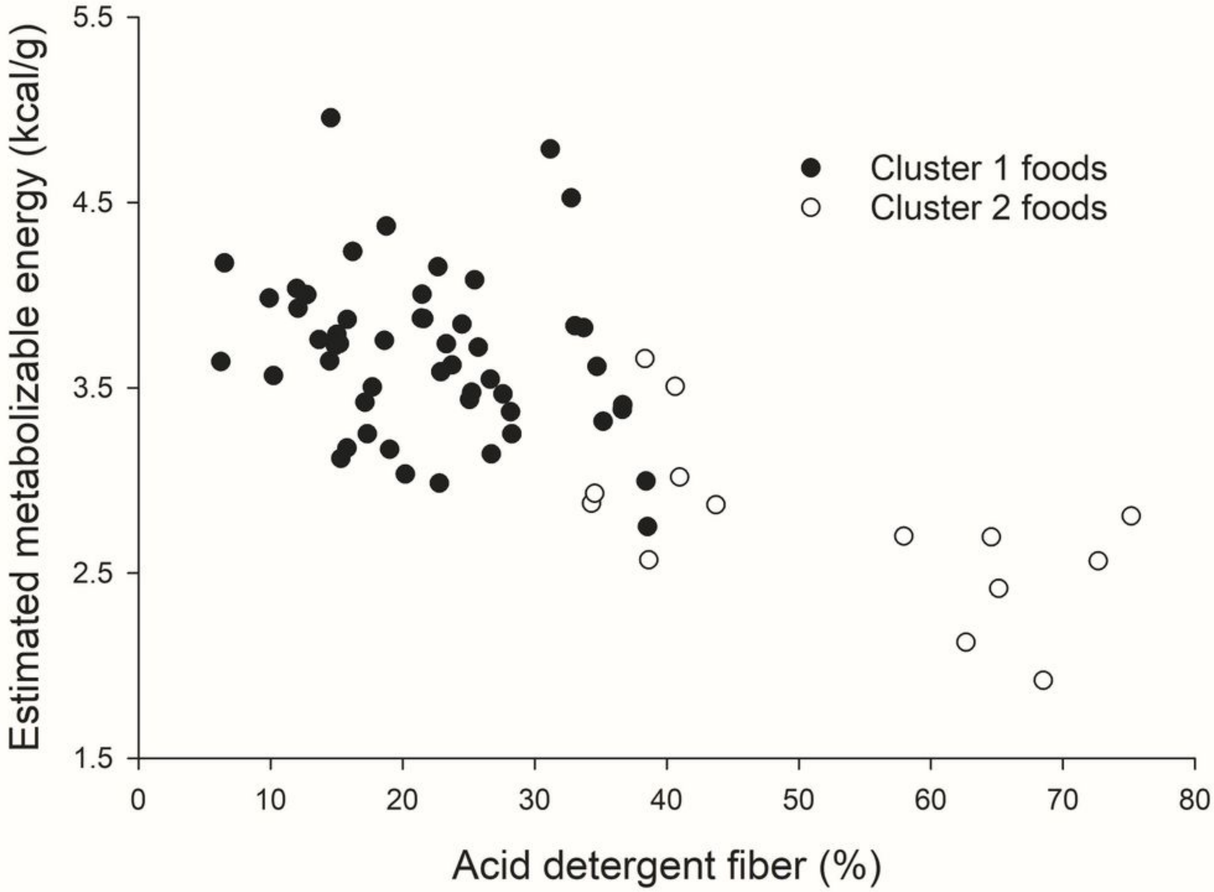

Figure 3

Relationships between metabolizable energy (ME) and acid detergent fiber (ADF) of foods consumed by lactating *P. coquereli* determined from PCA followed by cluster analysis

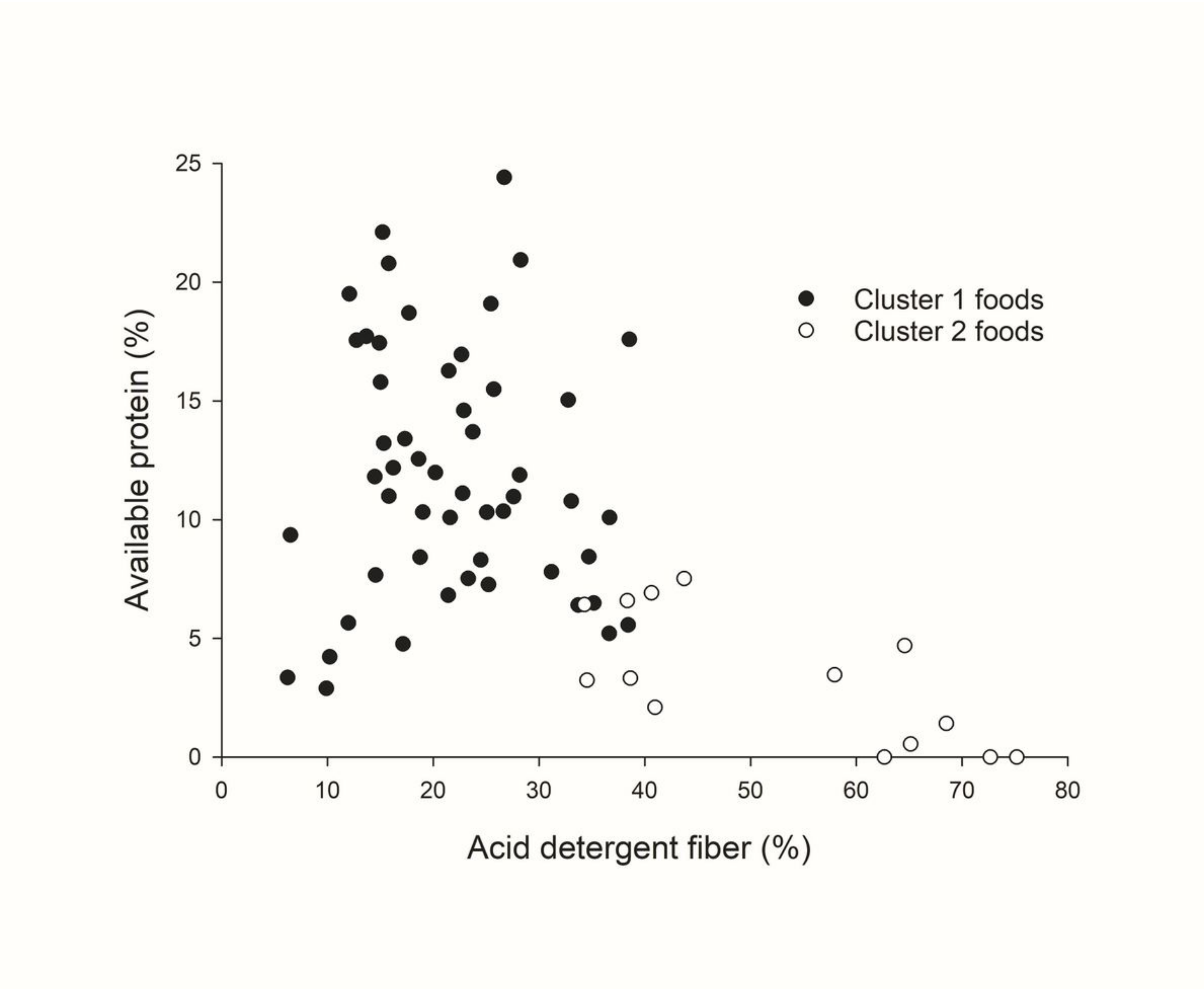

Figure 4

Relationship between available protein (AP) and acid detergent fiber (ADF) of foods consumed by lactating *P. coquereli* determined from PCA followed by cluster analysis