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Nutritional and Physiological Markers Are Mediated by Seasonality and Forest Access in Captive Coquerel's Sifakas

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ABSTRACT

Primates today live along a continuum of conditions in the wild and in captivity, providing diverse settings to study ecological flexibility. The western sifakas, a clade of endangered lemurs endemic to Madagascar, live in diverse ecosystems, including dry, spiny, and montane forests, and persist even in small forest fragments across their native ranges. A colony of the Coquerel's sifakas (*Propithecus coquereli*) is also housed at the Duke Lemur Center (DLC) in Durham, NC, where select groups gain access to forest enclosures, a setup that blends human management with an experimental introduction to non-native habitats. To better understand how sifakas adjust to local conditions, we profiled a suite of circulating nutritional and physiological markers collected from DLC sifakas with and without forest access in two seasons. In spring compared to summer, the sifakas had elevated concentrations of many amino acids, which may reflect the seasonal consumption of proteinaceous young leaves. Sifakas with forest access, compared to peers in stall enclosures, had elevated glucose, hippuric acid, the liver enzyme ALT, and a marker of muscle protein turnover—3-methylhistidine. These results likely differentially reflect freely foraged diets and the energy required for locomotion and thermoregulation under more naturalistic conditions. Calcium concentrations were well above values published for wild sifakas in Madagascar. Whereas some markers, like ALT, highlight how sifakas adjust to environmental heterogeneity seemingly without detrimental health effects, others, like calcium, may point to the potential consequences of a mismatch between the environments where species evolved to live and the conditions they now face. Our results can inform husbandry management and dietary optimization for this endangered species in captivity. More broadly, we advocate for research of captive wildlife under ecologically relevant conditions to inform understanding of how wild kin persist under diverse, novel, and local conditions, with implications for conservation management and monitoring in the wild.

1 | Introduction

Due to anthropogenic modification of Earth's ecosystems, wild primates today live along a continuum of conditions. These conditions range from pristine and contiguous habitats rich in endemic biodiversity to degraded, fragmented, agroforestry, and even urbanized ones teeming with introduced and invasive species (Chapman and Peres 2001, 2021; Eppley et al. 2015;

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Summary

Sifakas are endangered primates from Madagascar, where they live in many different types of forests. Some populations even persist in small forest fragments. A colony of Coquerel's sifakas is kept at the Duke Lemur Center in Durham, NC, where some animals gain access to expansive forest enclosures in which they roam and forage freely. This setup provides an opportunity to study how sifakas have adjusted to life in a non-native forest and can help us learn how wild sifakas may adjust following conservation interventions like reintroductions or translocations. We measured health markers collected from the sifaka colony in spring and summer, from animals with and without access to forests. In spring, compared to summer, the sifakas had greater concentrations of many amino acids, the building blocks of proteins, that may come from eating copious young leaves. Sifakas in forests, compared to peers in stalls, had greater concentrations of glucose, a liver enzyme, thyroid hormone, and a marker of muscle protein turnover, results that differently reflect freely foraged diets and the energy expended to roam and maintain stable body temperature under more naturalistic conditions. These results provide clues as to how sifaka bodies tolerate different diets and environments.

- **Practitioner Points**
  - We profiled nutritional and physiological markers in sifakas, endangered primates from Madagascar, managed in captivity in expansive, non-native forest enclosures.
  - Amino acids were greater in spring versus summer, likely reflecting seasonal young leaf consumption; forest access was associated with greater glucose, hippuric acid, ALT, 3-methylhistidine, and thyroxine, reflecting foraged diets and the energy required to maintain homeostasis under more naturalistic conditions.
  - Captive wildlife offers an opportunity to study ecological flexibility and resilience linked to local conditions with implications for conservation management.

Thatcher et al. 2023; Estrada et al. 2012). A key research focus in primatology is to study how primates can persist under diverse conditions and the associated consequences to individual and population-level health and reproduction. For example, previous and ongoing research of wild primates has examined abundance and density (Almeida-Rocha et al. 2017), ranging and behavioral flexibility (Irwin 2008; Ménard et al. 2013; Mclennan et al. 2017), markers of health, parasitism, and stress (Irwin et al. 2010; Gillespie and Chapman 2008; Thatcher et al. 2021; Kaisin et al. 2021), body size, condition, and morphology (Irwin et al. 2019; Altmann et al. 1993; Rayadin and Spehar 2015), differences in gut microbiome structure and function (Moy et al. 2023; Barelli et al. 2015; Amato et al. 2013), and changes to sociality and reproduction (Shil et al. 2020; Dunbar 1987), all linked to habitat quality. Moreover, primate conservation interventions, such as translocations or reintroductions, depend on determining species-specific limits of tolerable conditions (Franquesa-Soler et al. 2022), the individuals that are fit for release (Moore et al. 2014; Britt et al. 2004), and monitoring strategies to document how individuals and populations adjust and fare post-release (Strum 2005; Richard-Hansen et al. 2000; Tricone 2018; Donati et al. 2020; Day et al. 2009).

Populations of certain primates also live under various captive and semi-captive settings globally, including zoos, sanctuaries, and research facilities; others have been experimentally (or accidentally) introduced to non-native habitats (Jones et al. 2018), for example, the rhesus macaques of Cayo Santiago (Rawlins and Kessler 1986), that have been valuable resources for long-term and retrospective research in primatology (Widdig et al. 2016; Berman 2016; Francis and Wang 2025). Adding this spectrum of conditions to those under which primates naturally live can provide additional, rich opportunity to probe how primates can adjust to novel, local, and diverse conditions through behavior and physiology (Thompson et al. 2020; Evans 1989); determine how they impact non-native or regenerating ecosystems (Oliveira-Silva et al. 2018; Jones et al. 2018); to model how wild populations may cope with continued habitat change and extreme weather events (Francis and Wang 2025; Morcillo et al. 2020); and to identify consistent environmental features that support species-specific success.

The western sifakas (*Propithecus coronatus, Propithecus coquereli, Propithecus deckenii*, and *Propithecus verreauxi*) are a fascinating system for research of primate responses to habitat heterogeneity. These species comprise a clade of endangered lemurs from Madagascar that inhabit an array of forest ecosystems, including the dry deciduous and limestone forests of the west, spiny forests of the southwest, humid forests of the southeast, montane forests of the central highlands, and the transitional sites between these ecotypes (Wilmé and Callmander 2006; Randrianjaka et al. 2024; Blanco et al. 2023; Ramilison et al. 2021; Salmona et al. 2014; King et al. 2014; Rakotonirina et al. 2014). Populations also persist in numerous natural and/or anthropogenic forest fragments throughout the species' geographic ranges (Randrianjaka et al. 2024; Ramilison et al. 2021) and, in some places, are even frequently seen in villages dominated by introduced trees (Salmona et al. 2014). Sifakas are anatomical frugo-folivores (Campbell et al. 2000) and forage flexibly for a plant-based diet that varies seasonally and with local availability (Sato et al. 2016). Across seasons, sifakas tend to balance nutrient intake, and especially protein, by adjusting foraging time even at the cost of caloric intake (Irwin et al. 2015). In more anthropogenic habitats, their diets can include leaves, fruits, and flowers from introduced tree species like mango and tamarind (Salmona et al. 2014). Among lemurs, sifakas have a slow life history. This is particularly true of females that tend to have their first offspring later in life, live longer, and reproduce at later ages compared to other mammals of similar sizes, as strategies to endure environmental unpredictability and stochasticity (Richard et al. 2002).

A stable and breeding population of Coquerel's sifakas (*P. coquereli*) is also currently managed in captivity by the Duke Lemur Center (DLC) in North Carolina, USA. Founders of this population were brought into captivity in the 20th century for non-harmful research, and incremental advancements in veterinary and husbandry practices over the decades, including granting animals outdoor access to large forest enclosures, has improved population health, reproduction, and life expectancy (Hartng 1988; Zehr et al. 2014; Wright 2008). At the time of this study, DLC sifakas were socially housed in indoor/outdoor stalls and fed nutritious diets that included seasonal browse from local plants; most groups also gained unrestricted access to expansive forest enclosures (0.4–6.6 ha) when overnight temperatures reliably remained above 5°C, typically from April through October. This husbandry setup, which blends captive management with an experimental introduction to a non-native forest, provides an opportunity to study primate behavioral and ecological flexibility. To date, research on this colony has highlighted that captive sifakas introduced to non-native forests exhibit a behavioral repertoire that mimics that of their wild kin in native habitats, including occupying home ranges of similar sizes, traveling similar distances daily, and spending copious time foraging for a diverse, frugo-folivorous diet of seasonal and local fare (Greene et al. 2023; Abhau 2007). Like wild sifakas (Yamashita 2008), DLC sifakas forage for plants, and especially young leaves, with a high ratio of protein to fiber (Greene, Blanco, et al. 2024). To cope with fluctuating temperatures, DLC sifakas variably use huddling, sunning, and tree hugging (Figure 1), the latter being a behavior reported from western sifakas in Madagascar (Chen-Kraus et al. 2023). This behavioral adjustment to local conditions points to an interconnected suite of genetic, morphological, sensory, and digestive mechanisms that support ecological flexibility, including notable adaptations to folivory (Guevara, Greene, et al. 2021; Guevara, Webster, et al. 2021; Campbell et al. 2000; Greene et al. 2020). However, we currently lack understanding of the associated physiological and metabolic mechanisms, costs, and limits to this ecological flexibility, in large part, due to the challenges of identifying diagnostic markers for different primates and determining the range of normal values that reflect healthy adjustment to different conditions.

To begin filling this gap, we profiled a suite of nutritional, physiological, and metabolic markers from blood samples collected on the DLC sifaka colony during spring and summer in 2021, from groups with and without unrestricted access to forest enclosures. Our selected markers include circulating amino and carboxylic acids, electrolytes, glucose, cholesterol, and vitamin C, markers of liver and kidney function, blood cell counts, thyroid hormones, and a marker of muscle-protein turnover. Under the overarching hypothesis that behavioral and metabolic flexibility work in consort in primates to facilitate ecological flexibility linked to local conditions, we expect to find variation in our blood markers relative to the lemurs' seasonal diets and access to forests. For example, we expect greater signatures of protein and plant secondary compound intake in spring, when young and proteinaceous leaves dominate foraged and provisioned diets. We also expect markers of protein, tannins, sugar, and electrolytes to be elevated in sifakas with forest access linked to their greater foraging effort and food intake. We further expect sifakas with forest access to show greater signatures of muscle turnover and overall metabolism linked to their greater locomotor activity and thermoregulatory requirements. And lastly, we may pinpoint additional markers linked to the potential health consequences of environmental mismatch (Greene, Ellsaesser, et al. 2024), that is, of animals living under conditions in which they did not evolve, with implications both

FIGURE 1 | Photos of captive Coquerel's sifakas in the study, including in stall enclosures (left column) and forest enclosures (center and right columns), while eating provisioned or foraged browse (top row), and while resting, tree hugging, and locomoting (bottom row).

for the husbandry of captive sifakas and for predicting how wild kin may tolerate environmental heterogeneity and change.

2 | Methods

2.1 | Subjects, Blood Sampling, and Analyses

The subjects were 26, adult Coquerel's sifakas (14 females, 12 males), aged 3–25 years, that lived in 13 mixed-sex social groups of 2–4 individuals that variably included younger offspring (Table 1). Some females were reproductively intact, others were on hormonal contraception, per recommendations from the Species Survival Plan. All sifakas were healthy at the time of study, as assessed by the veterinary clinicians on our team (L. N. E. and C. V. W.). The animals received a once-daily diet of folivore chow (Mazuri 5672 Leaf Eater Mini Biscuit), a rotating blend of leafy greens, vegetables, beans, and nuts, and provisioned browse harvested from the local woods. The sifakas were maintained in large, indoor/outdoor stalls, and some groups gained unrestricted access to forest enclosures. Sifakas with forest access could roam and forage ad libitum for local vegetation on top of standard fare. Details on sifaka foraging and ranging ecology while in forest enclosures under this regimen were published previously (Greene et al. 2023). Clean water was always freely available.

We collected blood samples from subjects in the spring (May 11th to June 22nd) and summer (August 10th to September 8th) of 2021. In spring, we sampled all 26 subjects. At this time, 17 subjects lived in 8 social groups with access to forest enclosures; the remaining 9 sifakas resided in 5 social groups without forest access. In summer, due to a combination of colony turnover, illness, and animal exportation, we could only sample 18 sifakas. At this time point, 12 sifakas lived in 6 social groups with forest access; 6 lived in 4 social groups without forest access. Between the spring and summer sampling points, one social group gained forest access, and two social groups lost forest access, but otherwise all study animals remained under the same conditions throughout the study. All animals with forest access were experienced free-rangers, and no subject was introduced to a forest enclosure for the first time during this study.

During 10 days in spring and 8 days in summer, DLC veterinary staff collected blood samples from the femoral or saphenous vein of awake, manually restrained sifakas in the morning hours (8:30–11 a.m.) near their home enclosures. Groups in forest enclosures were free to forage until sampling, when DLC staff voluntarily locked up animals in either indoor housing units or covered forest shelters. Groups in stall enclosures had access to leftover dietary items from the previous day, but did not receive new daily rations until after sampling. After sampling, animals were immediately returned to their social groups and home enclosures. From the drawn whole blood, we placed 1 mL in EDTA in the fridge to be preserved for a rapid complete blood cell count (CBC). The remaining sample (~3 mL) was placed in serum separator tubes, allowed to clot at room temperature for 30 min, spun to serum, and aliquoted for a suite of analyses. One 750 µL aliquot was placed in the refrigerator for a rapid serum chemistry panel. The remaining serum aliquots were banked at −80°C.

We submitted blood samples for five different types of analyses (Table 2). Whole blood and serum were shipped on the day of sampling to IDEXX Laboratories Inc. (ME, USA) for a standard and rapid serum chemistry panel and CBC. We submitted 30 µL aliquots of serum from a subset of animals to the Duke Proteomics and Metabolomics Shared Resource core for a large metabolomics panel (Biocrates Q500 kit); 500 µL aliquots of serum to the Michigan State University Veterinary Diagnostic Laboratory for a thyroid panel (total triiodothyronine [T_3], total thyroxine [T_4], free T_4); and 200 µL aliquots of serum for all samples with enough leftover volume to Eurofins for vitamin C testing (Abcam Ascorbic Acid Assay Kit ab65656).

TABLE 1 | Study groups, composition, subjects, and details about housing at each sampling time point.

Group	Group composition[a]	Study subjects	Spring forest access	Summer forest access
Beatrice	1 F, 1 M	1 F, 1 M	Yes	Not sampled
Furia	1 F, 1 M	1 F, 1 M	Yes	Yes
Gertrude	1 F, 1 M	1 F, 1 M	Yes	Yes
Gisela	1 F, 1 M, 1 f, 1 m	1 F, 1 M	Yes	Yes
Lupicina	1 F, 1 M, 1 m	1 F, 1 M	Yes	Yes
Magdalena	1 F, 1 m	1 F	Yes	No
Pompeia	2 F, 1 M	2 F, 1 M	Yes	No
Rodelinda	1 F, 2 M	1 F, 2 M	Yes	Yes[b]
Bertha	1 F, 1 M	1 F, 1 M	No	Yes
Euphemia	1 F, 1 M	1 F, 1 M	No	Not sampled
Isabella	1 F, 1 M	1 F, 1 M	No	No
Justa	1 F, 1 M	1 F, 1 M	No	Not sampled
Valeria	1 F, 1 M	1 F	No	No
Total	31	26	17 yes; 9 no	12 yes; 6 no

[a] F/M refers to animals > 3 years of age; f/m refers to animals < 3 years of age.
[b] Only 1 M sampled in summer.

TABLE 2 | Number of sifakas contributing samples to each analysis type per season and housing condition.

Analysis	Spring		Summer	
	Forest access	No forest access	Forest access	No forest access
Total number of sifakas	17	9	12	6
Metabolomics panel	12	6	10	3
Serum chemistry panel	17	9	11	6
Complete blood cell count	17	9	12	5
Thyroid hormone panel	17	9	11	6
Vitamin C	9	5	11	2

2.2 | Statistical Analyses

We selected for statistical analyses a subset of analytes from the metabolomics, serum chemistry, and CBC panels which we predicted would relate to seasonal diets or forest access. For the metabolomics panel, we focused on 20 amino acids, three carboxylic acids (aconitic, hippuric, and lactic acids), and a marker of muscle protein turnover (3-methylhistidine) (Long et al. 1975; Neuhäuser and Bässler 1984). For the serum chemistry panel, we selected the electrolytes (sodium, potassium, chloride, calcium, and phosphorus), glucose, cholesterol, two liver markers (alanine transaminase [ALT] and aspartate transaminase [AST]), one kidney marker (blood urea nitrogen [BUN]), and two blood proteins (albumin and globulin). For the CBC panel, we selected total red blood cells, total white blood cells, and the relative proportion of lymphocytes, neutrophils, and monocytes. We retained three thyroid markers (total T_3, total T_4, free T_4) and vitamin C for statistics.

We performed all statistical analyses in RStudio (version 2023.12.0) (R CORE TEAM 2022) with the R software language (version 4.2.1) (Rstudio Team 2023). The data best fit the normal distribution. We therefore computed a single ANOVA per marker, including each analyte as the dependent variable, season (two categories: spring or summer), concurrent forest access (two categories: yes or no), sex (two categories: male or female), and age (continuous variable, in years) as the independent variables. We visually inspected the spread of data for outliers. For only two markers—ALT and BUN—did we visually identify a single outlying sample with elevated concentrations. We recomputed statistical analyses for these markers without this sample to ensure that the results were not biased. We report results from the full models herein.

3 | Results

We found strong effects of both seasonality and forest access on different subsets of nutritional and physiological markers in Coquerel's sifakas. The amino acids were associated with season (Table 3). The sums of the essential (Figure 2a), non-essential (Figure 2b), aromatic (Figure 2c), and branched-chain (Figure 2d) amino acids were all greater in spring compared to summer, as were concentrations of 11 out of 20 individual amino acids. We note particularly strong patterns for the three branched-chain amino acids—isoleucine (Figure 2e), leucine (Figure 2f), and valine (Figure 2g). No single amino acid, nor the summation of various amino acid categories, varied in concentration with forest access. We found only the methionine concentration to be correlated with sex and was greater in males compared to females ($F_{1,26} = 4.562$, $p = 0.042$). Age was inversely correlated to concentrations of cystine ($F_{1,26} = 4.815$, $p = 0.037$), leucine ($F_{1,26} = 5.527$, $p = 0.027$), and valine ($F_{1,26} = 8.550$, $p = 0.007$), such that older animals had lower circulating concentrations.

In contrast, we found no effect of season on any carboxylic acid, but we did find effects of forest access. Sifakas with forest access versus those without forest access had significantly greater concentrations of hippuric acid ($F_{1,26} = 8.718$, $p = 0.007$; Figure 3a), though they had similar concentrations of aconitic acid ($F_{1,26} = 3.587$, $p = 0.069$; Figure 3b) and lactic acid ($F_{1,26} = 0.003$, $p = 0.954$). Aconitic acid varied by age ($F_{1,26} = 4.616$, $p = 0.041$), with older animals having lower concentrations. We found no other relationships between animal sex or age and any carboxylic acid.

We likewise found that forest access, but not season, was related to serum chemistry and CBC markers (Table 4). Sifakas with forest access, compared to those without forest access, had significantly lower circulating chloride (Figure 4a) and phosphorus (Figure 4b) concentrations. Calcium was not significantly different between housing conditions; however, concentrations across all samples averaged 13.2 mg/dL and varied from 9.7 to 20.02 mg/dL. We note that concentrations from 10 of these samples were between 14.4 and 20.2 mg/dL, which is above in-house reference intervals for this species in captivity (8.1–14.3 mg/dL).

Glucose (Figure 4c) and ALT (Figure 4d) were both elevated in sifakas with forest access compared to those without. The sifakas with forest access also had a greater proportion of lymphocytes (Figure 4e) and a reduced proportion of neutrophils (Figure 4f) among their white blood cells. Only phosphorus showed a relationship with season, being more concentrated in spring versus summer. We found no other effects of forest access or season on other serum chemistry or CBC markers, nor did we find any effects of season or housing on circulating vitamin C.

As expected, we found relationships between animal sex and age on serum chemistry and CBC markers. Compared to females, males had significantly lower concentrations of chloride ($F_{1,38} = 10.232$, $p = 0.003$) and cholesterol ($F_{1,38} = 4.142$, $p = 0.049$). Males had greater concentrations of white blood cells

TABLE 3 | Results of amino acids relative to seasonality and forest access.

		Season		Forest access	
Amino acid (AA)	**Direction**	**$F_{1,26}$ value**	***p*-value**	**$F_{1,26}$ value**	***p*-value**
Alanine	No trend	1.895	0.180	0.252	0.620
Arginine	**Spring > summer**	**4.107**	**0.053**	0.969	0.339
Asparagine	No trend	0.000	0.984	3.140	0.088
Aspartic acid	**Spring > summer**	**8.749**	**0.007**	0.719	0.404
Cysteine	No trend	0.084	0.774	1.472	0.236
Glutamine	No trend	0.198	0.660	0.106	0.748
Glutamic acid	**Spring > summer**	**5.687**	**0.025**	0.072	0.791
Glycine	**Spring > summer**	**5.427**	**0.028**	1.260	0.272
Histidine	No trend	1.324	0.260	0.029	0.866
Isoleucine	**Spring > summer**	**15.384**	**< 0.001**	0.838	0.368
Leucine	**Spring > summer**	**13.902**	**< 0.001**	2.460	0.129
Lysine	**Spring > summer**	**6.991**	**0.014**	0.358	0.555
Methionine	No trend	0.765	0.390	0.029	0.867
Phenylalanine	**Spring > summer**	**8.205**	**0.008**	1.275	0.269
Proline	No trend	1.047	0.316	3.465	0.074
Serine	No trend	2.427	0.131	0.002	0.963
Threonine	No trend	1.760	0.196	1.110	0.302
Tryptophan	**Spring > summer**	**6.293**	**0.019**	0.561	0.461
Tyrosine	**Spring > summer**	**4.798**	**0.038**	1.604	0.217
Valine	**Spring > summer**	**15.115**	**< 0.001**	3.309	0.080
Sum of essential AAs	**Spring > summer**	**12.476**	**0.002**	0.152	0.699
Sum of non-essential AAs	**Spring > summer**	**4.559**	**0.042**	0.025	0.876
Sum of aromatic AAs	**Spring > summer**	**8.434**	**0.007**	1.643	0.211
Sum of branched-chain AAs	**Spring > summer**	**16.545**	**< 0.001**	2.645	0.116
Sum of all AAs	**Spring > summer**	**6.722**	**0.015**	0.049	0.826

Note: Bold values in the table shows the result was significant if *p* was greater than or equal to 0.05.

overall ($F_{1,38} = 3.922$, $p = 0.055$), but a decreased proportion of lymphocytes compared to females ($F_{1,38} = 4.628$, $p = 0.038$). We detected a positive relationship between animal age and concentrations of sodium ($F_{1,38} = 4.073$, $p = 0.051$), chloride ($F_{1,38} = 7.560$, $p = 0.009$), ALT ($F_{1,38} = 4.765$, $p = 0.035$), and AST ($F_{1,38} = 5.438$, $p = 0.025$), and a negative relationship between animal age and albumin ($F_{1,38} = 4.262$, $p = 0.046$). Among white blood cells, the proportion of lymphocytes ($F_{1,38} = 9.839$, $p = 0.003$) and monocytes ($F_{1,38} = 5.329$, $p = 0.027$) decreased with age, while the proportion of neutrophils increased ($F_{1,38} = 12.278$, $p = 0.001$). Vitamin C did not vary by animal sex or age.

Our marker of muscle protein turnover, 3-methylhistidine, was more concentrated in sifakas that had access to forest enclosures compared to those without forest access ($F_{1,26} = 6.654$, $p = 0.016$; Figure 5a) but was unrelated to season ($F_{1,26} = 1.256$, $p = 0.273$). Total T_3 was correlated neither to forest access ($F_{1,26} = 0.804$, $p = 0.375$; Figure 5b) nor season ($F_{1,26} = 0.018$, $p = 0.894$); however, sifakas with forest access had significantly greater values of total T_4 ($F_{1,26} = 10.624$, $p = 0.002$; Figure 5c) and free T_4 ($F_{1,26} = 12.293$, $p = 0.001$; Figure 5d) compared to peers without forest access. Neither total nor free T_4 differed seasonally (TT_4: $F_{1,26} = 1.497$, $p = 0.229$; FT_4: $F_{1,26} = 0.702$, $p = 0.407$). We detected no difference in any of these markers of energetics by animal sex or age.

4 | Discussion

Considering captivity as a type of anthropogenic habitat, we studied a managed population of Coquerel's sifakas to determine how seasonality and habitual forest access mediate markers of nutrition and physiology. In spring compared to summer, regardless of housing condition, the sifakas had markedly elevated concentrations of many circulating amino acids, especially the three branched-chain amino acids. When granted access to forest enclosures, the sifakas had reduced concentrations of chloride and phosphorus, but greater concentrations of glucose, hippuric acid, the liver enzyme ALT, the marker of muscle-protein turnover 3-methylhistidine, total and free T_4, and a greater ratio of lymphocytes to neutrophils. We posit that seasonal swings in amino acids may be linked to the seasonal availability of foraged and/or provisioned young leaves. The suite of markers that varied with forest access may

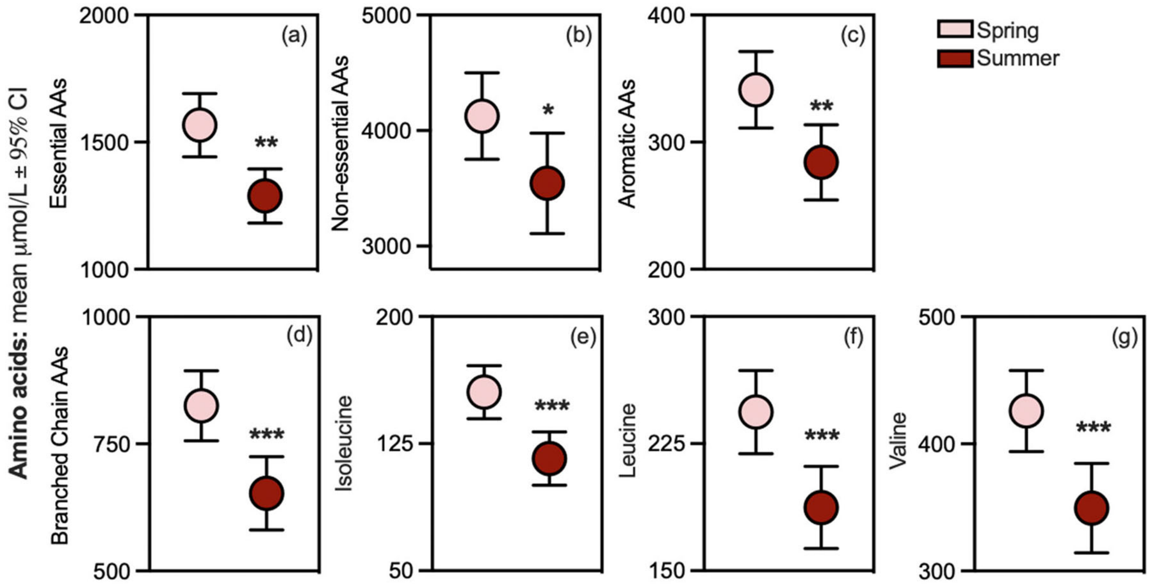

FIGURE 2 | Variation in circulating amino acids relative to season in captive Coquerel's sifakas. Depicted here are the mean ± 95% CI concentrations of the summation of all (a) essential, (b) non-essential, (c) aromatic, and (d) branched-chain amino acids, as well as concentrations of each branched-chain amino acid, notably (e) isoleucine, (f) leucine, and (g) valine, for all subjects sampled in spring (pink) and summer (burgundy). *p 0.05; **$p < 0.01$; ***$p < 0.001$.

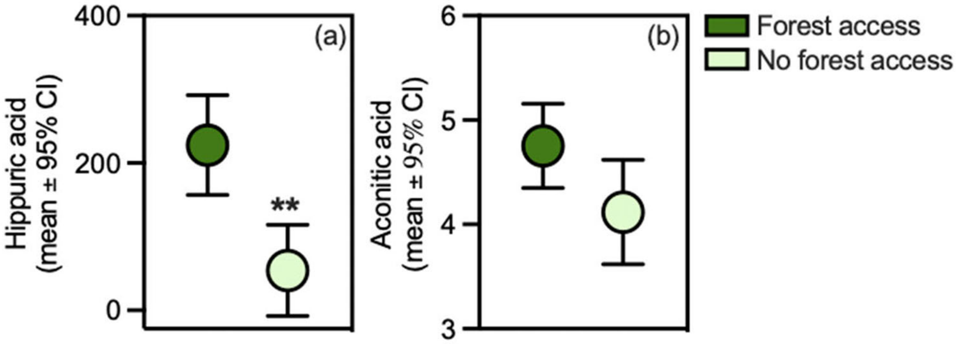

FIGURE 3 | Variation in circulating carboxylic acids relative to forest access in captive Coquerel's sifakas. Depicted here are the mean ± 95% CI concentrations of (a) hippuric and (b) aconitic acids for all subjects with access to forest enclosures (dark green) versus without access to forest enclosures (light green). **$p < 0.01$.

differentially reflect freely foraged diets, changes to immune balance, and the energy required for digestion, locomotion, and thermoregulation under more naturalistic conditions. Our results underscore the behavioral, ecological, metabolic, and physiological flexibility of the Coquerel's sifaka under human care (Greene et al. 2023), with implications for understanding how wild populations may cope with continued habitat modification and climate change.

Regardless of forest access, the sifakas had elevated concentrations of many amino acids in spring compared to summer. The patterns were particularly strong for the essential amino acids, aromatic amino acids, and most notably for the branched-chain amino acids. These categories are not mutually exclusive: All three branched-chain (isoleucine, leucine, valine) and two aromatic (phenylalanine and tryptophan) amino acids are essential amino acids and must be obtained from dietary sources (Hou and Wu 2018) or via gut-microbial production (Gill et al. 2006). One explanation for these seasonal patterns relates to the seasonal consumption of young leaves. Sifakas without forest access are provisioned daily with local browse cut fresh by husbandry staff. In spring versus summer, provisioned browse is dominated by young versus mature leaves. Sifakas with forest access spend significantly more time foraging for young leaves in spring and mature leaves in summer (Greene et al. 2023). Foraged and provisioned young leaves, compared to mature leaves, have double the ratio of available protein to fiber on average (Greene, Blanco, et al. 2024), and available protein in provisioned browse decreases significantly from the first to the second half of the free-ranging season (Campbell et al. 2001). For many wild primates, including sifakas, seasonal diets are common (Sato et al. 2016; Lambert and Rothman 2015); however, folivorous lemurs generally balance nutrient intake, and especially protein, by adjusting caloric consumption (Irwin et al. 2015; Dröscher et al. 2016; Yamashita 2008) and/or by foraging for

TABLE 4 | Results of serum chemistry, vitamins, and CBC markers relative to season and forest access.

		Season		Forest access	
Serum chemistry/CBC marker	**Direction**	**$F_{1,38}$ value**	**p-value**	**$F_{1,38}$ value**	**p-value**
Sodium	No trend	0.367	0.548	1.274	0.266
Potassium	No trend	0.543	0.466	0.724	0.400
Chloride	**Forest access < no access**	0.511	0.479	**9.324**	**0.004**
Calcium	No trend	0.340	0.563	3.612	0.065
Phosphorous	**Spring > summer; forest access < no access**	**4.747**	**0.036**	**11.958**	**0.001**
Glucose	**Forest access > no access**	1.450	0.236	**6.768**	**0.013**
Cholesterol	No trend	0.393	0.534	2.678	0.110
ALT	**Forest access > no access**	0.019	0.891	**9.270**	**0.004**
AST	No trend	0.117	0.735	2.437	0.127
BUN	No trend	0.778	0.383	0.181	0.673
Albumin	No trend	0.166	0.686	0.279	0.601
Globulin	No trend	1.049	0.313	0.026	0.873
Red blood cells	No trend	0.182	0.672	0.427	0.517
White blood cells	No trend	0.029	0.865	2.105	0.155
Lymphocytes %	**Forest access > no access**	2.202	0.146	**5.531**	**0.024**
Neutrophils %	**Forest access < no access**	1.274	0.266	**5.879**	**0.020**
Monocytes %	No trend	1.279	0.265	0.702	0.407
Vitamin c	No trend	0.010	0.921	0.586	0.452

Note: Bold values in the table shows the result was significant if *p* was greater than or equal to 0.05.

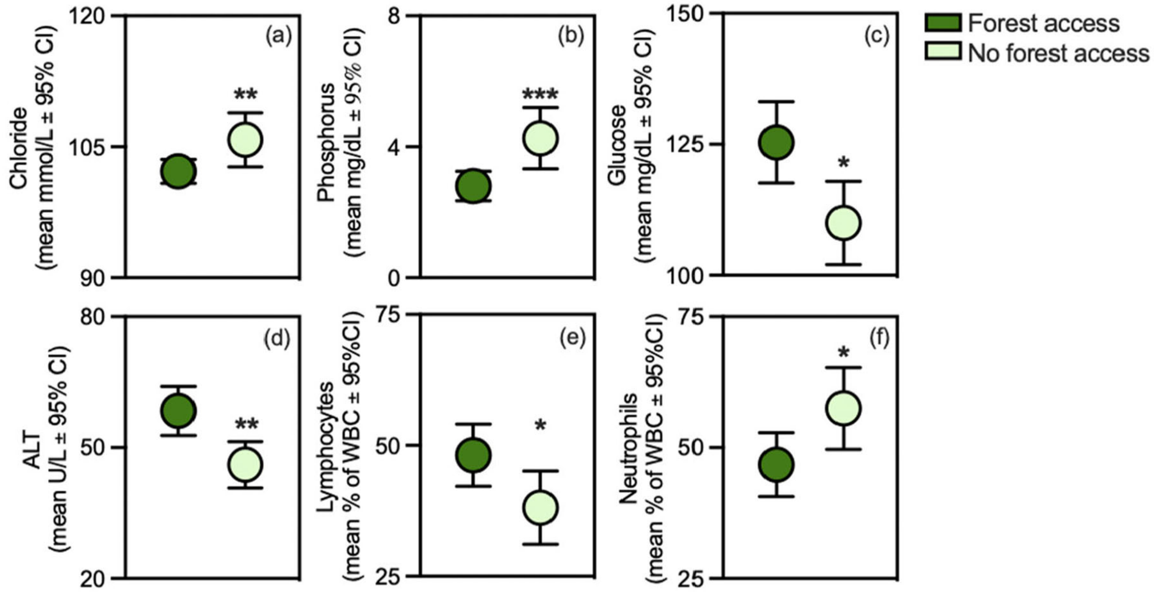

FIGURE 4 | Variation in circulating serum chemistry markers relative to forest access in captive Coquerel's sifakas. Depicted here are the mean ± 95% CI concentrations (a) chloride, (b) phosphorus, (c) glucose, (d) alanine transaminase (ALT), as well as the percentage of white blood cells that were (e) lymphocytes and (f) neutrophils, for all subjects with access to forest enclosures (dark green) versus with no access to forest enclosures (light green). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p \leq 0.001$.

particular food items (Veilleux et al. 2024). For sifakas, an intrinsic balance of protein to calories may be tuned to the low-protein nature of Malagasy fruits and the subsequent folivory used to meet protein requirements (Donati et al. 2017). If so, sifakas living under high-protein conditions like captivity may still retain the instinct to select for protein-rich young leaves. Future studies could profile the amino acids in the sifakas' foraged fare and determine nutrient balance across seasons,

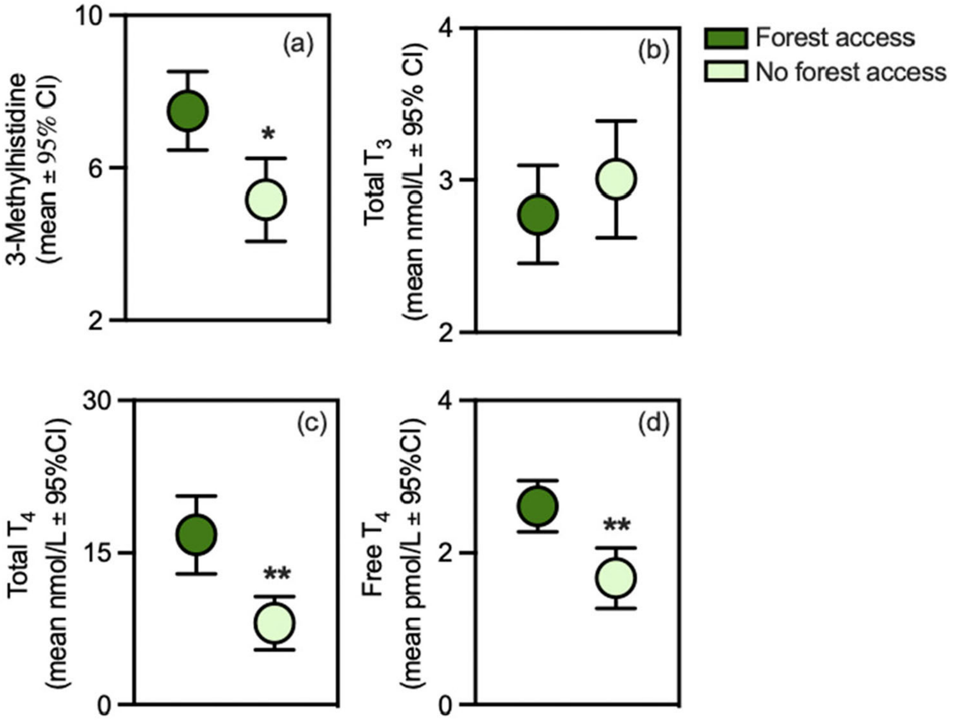

FIGURE 5 | Variation in circulating markers of physiology and energy expenditure relative to forest access in captive Coquerel's sifakas. Depicted here are the mean 95% CI concentrations of (a) 3-methylhistidine, (b) total triiodothyronine (T_3), (c) total thyroxine (T_4), and (d) free thyroxine (T_4) for all subjects with access to forest enclosures (dark green) versus those with no access to forest enclosures (light green). $*p < 0.05$; $**p < 0.01$.

including those linked to browse consumption. If high protein intake in spring is of clinical concern, veterinary teams may consider seasonally adjusting the protein content of chow to account for that in consumed browse.

For sifakas with forest access, foraging behavior may explain some of our other findings. Notably, sifakas with forest access were free to forage up until sampling, whereas sifakas in stall enclosures did not receive provisioned diets until afterward. This difference in the timing of foraging likely explains the increased circulating glucose among forest dwellers. The greater hippuric acid among forest dwellers likely owes to the ingestion of plant secondary compounds, potentially including those in the hours before sampling (Rodriguez-Mateos et al. 2013). Derived from phenolics, hippuric acid in humans increases with fruit and vegetable consumption (Krupp et al. 2012; de Mello et al. 2017) and is correlated to fasting glucose (de Mello et al. 2017). In our study, increased circulating hippuric acid in forest-dwelling sifakas likely owed to a foraged diet rich in leaves, fruits, nuts, and flowers (Greene et al. 2023). The corresponding increases in the liver enzyme ALT could also reflect the increased work by the liver to metabolize and detoxify plant toxins (Liska 1998). Importantly, ALT in our study fell within reference ranges for wild sifakas (Irwin et al. 2010; Junge and Louis 2005; Page-Karjian et al. 2021; Rasambainarivo et al. 2014; Garell and Meyers 1995) and did not approach values that would be of clinical concern.

Dietary differences may also explain the elevated calcium in some individual sifakas. In Madagascar, average calcium values for wild sifaka species range from 9.8 to 11.9 mg/dL (Irwin et al. 2010; Garell and Meyers 1995; Page-Karjian et al. 2021; Rasambainarivo et al. 2014; Junge and Louis 2005) and maximum reported values for individuals within populations are 11.3–13.2 mg/dL (Page-Karjian et al. 2021; Rasambainarivo et al. 2014; Junge and Louis 2005). For sifakas in our study, the average calcium was 13.2 mg/dL, and our maximum value was 20.2 mg/dL. Like protein, forests in Madagascar may present a particular challenge for lemurs in obtaining calcium. Calcium in the foraged diets of wild lemurs, including sifakas, is lower compared to that in the diets of other primates (Irwin et al. 2017). If primate evolution in low-calcium environments selected for lemurs to be efficient at calcium uptake, life in high-calcium environments such as captivity could lead to mineral imbalance and pervasive hypercalcemia (Irwin et al. 2017). In winter, calcium values for DLC sifakas average around 11.0 mg/dL (L. K. G., unpublished data), highlighting that hypercalcemia in this population could be seasonal and reversible. Future analyses of ionized calcium, the biologically active form, and vitamin D could help determine if these elevated concentrations are of clinical concern. We encourage veterinary teams managing sifakas to consider adjusting provisioned chow that is currently enriched with calcium and vitamin D that stimulates calcium absorption. It would also be beneficial to determine calcium and vitamin D concentrations in foraged and provisioned browse relative to the foraged fare of wild Coquerel's sifakas.

When housed in forest enclosures, DLC sifakas spend considerable effort locomoting and thermoregulating, in addition to foraging. Indeed, forest-dwelling sifakas at the DLC, on average, spend about a quarter of their time foraging, maintain 3-day home ranges over 1 ha, and travel about 500 m/day (Greene et al. 2023).

They cope with nighttime ambient temperatures as low as 5°C and daytime ambient temperatures that soar above 30°C. Forest access thus challenges captive sifakas to express a fuller range of their behavioral and metabolic potential, which is what is likely reflected in their elevated concentrations of 3-methylhistidine, a marker of muscle protein turnover, and T_4, the major and more stable form of circulating thyroid hormone. Curiously, forest access was also associated with a reduced proportion of neutrophils and a greater proportion of lymphocytes, two types of white blood cells implicated in immunity. Lymphocytes primarily produce antibodies to combat pathogens, whereas neutrophils directly kill pathogens and are gaining recognition for their role in the inflammatory process and autoimmunity (Kubes 2018). We previously suggested that captive sifakas may experience persistent, low-grade inflammation linked to low circulating cortisol (Greene, Ellsaesser, et al. 2024) that could be driven by neutrophil activity. Perhaps forest access, while reducing the lifetime stress inherent to captivity (Seeley et al. 2021), especially for male sifakas (Seeley 2022), also reduces inflammation. Alternatively, perhaps sifakas in stall enclosures are paradoxically exposed to more environmental pathogens through proximity to human caretakers and pest animals, with corresponding increases in neutrophils. Taken together, these markers provide clues as to how differences in habitat may mediate differences in animal physiology, with implications for animal health and husbandry management.

Ultimately, we posit that captive primates under diverse environmental conditions provide under-tapped resources and living experiments for probing the proximate mechanisms that enable adjustment to local conditions. For Coquerel's sifakas, members of our team have documented foraging and ranging behavior (Greene et al. 2023), macronutrient selection (Greene, Blanco, et al. 2024), gut microbiome composition (Greene et al. 2018, 2021), cortisol dynamics (Greene, Ellsaesser, et al. 2024), and now markers of physiology and nutrition to demonstrate how primates native to the dry deciduous forests of northwest Madagascar have adjusted to human management in the piedmont forests of North Carolina. Some markers, like thyroid hormones, ALT, and hippuric acid, highlight how sifakas tolerate environmental heterogeneity seemingly without detrimental health effects. By contrast other markers, like the amino acids, calcium, neutrophils, and cortisol (Greene, Ellsaesser, et al. 2024) may instead point to the potential consequences of mismatch between the environments where species evolved to live and the conditions they now face. Additional research of this colony to analyze related markers (like vitamin D that is linked to calcium) and the composition of foraged foods (like plant secondary compounds that are linked to leaf-protein availability) could yield a more comprehensive understanding of the processes that underlie our results and their potential health implications. Continuing this line of research could inform management in captivity for this Critically Endangered species.

Future studies could also beneficially profile similar markers in populations of western sifakas across a gradient of habitat types and conditions, thereby building upon foundational work on sifakas by the Prosimian Biomedical Survey Project (Junge and Louis 2005; Page-Karjian et al. 2021; Rasambainarivo et al. 2014). This is especially important throughout Western Madagascar, where native dry forests face ongoing and significant threats (Waeber et al. 2015) and are slow to regenerate (Culbertson et al. 2022). Determining whether and how sifakas persist in degraded, mosaic, transitional, and regenerating ecosystems has clear implications for conservation management. More broadly, understanding the breadth of tolerable conditions for endangered wildlife and the species-specific metabolic processes that enable ecological flexibility may be key to managing future interventions such as reintroductions and translocations, and to predicting how at-risk populations will cope with ongoing and future habitat change.

Author Contributions

Lydia K. Greene: conceptualization, investigation, funding acquisition, writing – original draft, methodology, visualization, formal analysis. **Laura N. Ellsaesser:** conceptualization, investigation, methodology, writing – review and editing. **Cathy V. Williams:** conceptualization, investigation, methodology, writing – review and editing. **Claudia Wrampelmeier:** data curation. **Anne D. Yoder:** funding acquisition, writing – review and editing, supervision. **Marina B. Blanco:** conceptualization, investigation, funding acquisition, writing – original draft, visualization.

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Ethics Statement

This study was approved by Duke University's Institutional Care and Use Committee (Protocol #A039-21-02).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw data are available in the supporting material file S1.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
S1 Raw Data.