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Untargeted Serum Metabolomics of Feline Sporotrichosis Reveals Metabolic Changes Associated with Antifungal Treatment

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Abstract

Feline sporotrichosis is an emerging zoonosis caused by fungi of the genus *Sporothrix* and is notable for the high fungal burden in cutaneous lesions and the risk of direct transmission to humans. This longitudinal study characterized serum metabolic changes in seven naturally infected cats at baseline (T0) and after 60 days of itraconazole-based treatment (T60). Three healthy cats were clinically characterized but were not included in the metabolomic model. AST, ALT, GGT, and creatinine increased and hematocrit decreased at T60, while all treated cats showed clinical improvement. Docosane, pentacosane, dodecane, and 3-ethyl-2,6,10-trimethylundecane were the principal T0 discriminants (VIP ≥ 1.6), whereas tetrahydrolavandulol, 1-tetradecene, L-iditol, myo-inositol, and other metabolites were more abundant at T60. PLS-DA distinguished the two time points ($R^2Y = 0.974$; $Q^2 = 0.943$), with the temporal separation supported by leave-one-animal-out cross-validation and exact paired permutation testing ($p = 0.016$). The decrease in the four alkanes was consistent with the attenuation of oxidative processes described in the literature, although oxidative stress biomarkers were not measured. These findings identify a treatment-associated shift in the serum metabolic profile and candidate metabolites for validation in expanded cohorts.

Introduction

Sporotrichosis is a subcutaneous mycosis caused by fungi of the genus Sporothrix, with Sporothrix brasiliensis being the most virulent species in Brazil. This zoonosis has generated growing concern because of its high incidence in felines and the potential for transmission to humans, especially in urban areas with large concentrations of stray animals (Fiocruz, 2023; Barros et al., 2011). Since 1998, the continuous increase in human cases has contributed to the recognition of this condition as an emerging public health problem in Brazil (Barros et al., 2010). The disease is characterized by polymorphic lesions of the skin and subcutaneous tissue, caused by dimorphic and geophilic fungi of the Sporothrix schenckii complex (Almeida-Paes et al., 2017). In felines, sporotrichosis can present as localized cutaneous, lymphocutaneous, or, in more severe cases, systemic infection, the latter frequently associated with respiratory compromise and hematogenous dissemination, making therapeutic management more complex and the prognosis guarded (Gremião et al., 2021). S. brasiliensis has adapted to the urban environment in Brazil since the late 1990s, initially concentrated in Rio de Janeiro and now present across multiple states (Almeida-Paes et al., 2024). In Espírito Santo, the state where this study was conducted, human sporotrichosis became compulsorily notifiable by Ordinance No. 054-R/2020 and animal sporotrichosis by Ordinance No. 115-R/2022 (Espírito Santo, 2024). Between 2020 and October 2023, 2,910 human and 6,688 animal cases were notified in the metropolitan health region alone, with cats as the main vectors (Espírito Santo, 2023). For every two to three infected cats, one related human case is observed. These data illustrate the veterinary-public health interface of feline sporotrichosis and the need to better characterize the systemic impact of infection and treatment in this species. Metabolomic analysis can characterize systemic responses to infection and treatment and identify candidate markers for subsequent validation.

Unneutered mixed-breed cats with free outdoor access play a fundamental role in maintaining and propagating sporotrichosis in urban environments (Gremião et al., 2021). The purpose of metabolomics is to identify and quantify small molecules in biological samples, providing a systematic approach for characterizing the metabolic changes associated with diverse pathophysiological conditions. In the study of infectious diseases such as sporotrichosis, this approach offers a detailed view of animal metabolism that allows the monitoring of metabolic alterations across time points (Weiss; Wardrop, 2010; Rivera-Vélez et al., 2021). The metabolites analyzed in metabolomics are low-molecular-weight molecules, generally with masses up to 1500 Da, detectable in serum, plasma, urine, cerebrospinal fluid, saliva, tissues, and feces, representing intermediate or final products of amino acid, carbohydrate, lipid, vitamin, and mineral metabolism (Wishart, 2008; Canuto et al., 2018).

Feline sporotrichosis, by compromising different tissues and systems, has the potential to provoke systemic metabolic alterations detectable by mass spectrometry techniques, which are becoming increasingly accessible in research centers (Fiehn, 2002; Dunn; Ellis, 2005). Metabolomics has an established role in translational veterinary medicine, with the purpose of identifying biomarkers and characterizing the pathological mechanisms of disease (Rivera-Vélez et al., 2021). In this context, the present study clinically characterized healthy and infected cats and evaluated serum metabolic changes in seven infected cats between T0 and T60 using GC-MS and PLS-DA.

Materials and Methods

Ethical Approval and Study Design

This was a non-randomized longitudinal study of naturally infected cats followed from baseline to 60 days of treatment, together with a descriptive group of healthy cats. The healthy animals were clinically characterized but were not included in the PLS-DA or paired inferential analyses. The study was conducted in accordance with the ethical principles of animal experimentation, with approval from the Animal Use Ethics Committee of the Universidade Federal do Espírito Santo (CEUA Alegre; protocols No. 0007/2025 and 0008/2025). The owner informed consent form is provided as Supplementary Form S1.

Animal Selection

A total of 35 feline candidates were screened for study participation, with open lesions and known contact with already contaminated animals and/or residents of areas with high disease incidence, identified by tutors in the municipality of Jerônimo Monteiro, ES. Of these animals, 27 were diagnosed with sporotrichosis based on their clinical and epidemiological history, direct cytology, and observation of pleomorphic yeasts. In three individuals with low fungal burden on slide reading, samples were seeded on Sabouraud dextrose agar with chloramphenicol and incubated at 25°C, with readings taken after 15 days. The fungus was identified by color, texture, and colony morphology, followed by microscopic reading.

After the initial eligibility assessment, 11 infected animals underwent FIV/FeLV testing using the SensPERT FeLV Ag/FIV Ab Test Kit (Dechra®) performed on whole blood by immunochromatography. Four animals tested positive for one or both infections and were excluded, leaving seven infected animals in the study. Additional eligibility exclusions were based on age outside the range of one–five years, pre-existing hepatic or renal disease, inability of the owners to comply with follow-up, or behavior that prevented safe handling. Three clinically healthy animals that met the same eligibility criteria were included in the descriptive control group.

Animals were assigned to three clinical treatment categories. The healthy control category comprised three clinically healthy cats that received no antifungal treatment. The itraconazole-only category comprised three infected cats with no more than two cutaneous lesions, without disseminated lesions or respiratory compromise. The combination-treatment category comprised four infected cats with more than two lesions, disseminated lesions, or respiratory compromise; these animals received itraconazole combined with potassium iodide. All seven infected animals completed the 60-day follow-up and showed clinical improvement.

Antifungal Treatment

Itraconazole was administered orally at a fixed dose of 100 mg per cat every 24 h for 60 days to all seven infected cats. The four cats in the combination treatment category additionally received potassium iodide orally at 5 mg kg^{-1} every 24 h for 60 days. The doses were not changed during the 60-day study period of the present study. No treatment interruptions were reported, and the owners reported complete adherence to the prescribed regimens. Two cats continued itraconazole for an additional 60 days after T60; this extended treatment occurred after the analytical endpoint and was not included in the T0–T60 comparison.

Follow-up and Clinical Monitoring

Animals were evaluated and clinically monitored weekly. Complementary examinations and serum collection were scheduled at T0 (baseline), T30 (30 days), and T60 (60 days). The analyses reported in the present study were restricted to T0 and T60 because these were the time points available in the final paired dataset. Weekly monitoring records are provided in Supplementary Table S1.

Diet and Housing Management

Each cat remained on its habitual diet throughout the study and avoided the introduction of dietary changes during the treatment period. Serum collection was preceded by a standardized 12-hour fasting period. For the T0–T60 comparison, the paired longitudinal design allowed each cat to serve as its own reference, thereby reducing the influence of stable individual dietary differences on the observed temporal changes. Dietary origin was considered specifically when interpreting chlorogenic acid levels.

In positive cases of sporotrichosis, animals were isolated from other household individuals and the community throughout treatment until complete clinical remission of lesions. Clinical handling followed the Cat Friendly protocol (Carney et al., 2012).

Blood Collection and Laboratory Tests

Blood samples were obtained aseptically from the jugular (preferentially) or cephalic veins and placed in EDTA tubes for complete blood count and platelet count, and in coagulation activator gel tubes for serum biochemistry and metabolomics. Animals were fasted for approximately 4 h before sample collection for CBC and 12 hours for other samples. CBC and platelet count were performed using a Mindray BC-5000 Vet automated hematology analyzer. Serum biochemical measurements of urea, creatinine, AST, ALT, and GGT were performed using a FUJI DRI-CHEM NX500i analyzer (Fujifilm) by colorimetry at the Probiovet Veterinary Clinical Analysis Laboratory, Cachoeiro de Itapemirim, ES. All measurements were performed at T0 and T60.

Cytological Evaluation of Lesions

Material collections were performed from cutaneous lesions of the 35 suspect felines by direct exudate smear or imprint technique. For each animal, three to five slides per lesion were prepared, left to air-dry, and stained with Rapid Panoptic (Laborclin®), a Romanowsky-derived stain. Microscopic analysis was performed using a basic achromatic binocular microscope (Olen®) with 4×, 10×, 40×, and 100× (oil-immersion) objectives, to identify structures compatible with *Sporothrix* spp.

Fungal Culture

For mycological confirmation of suspicious cases with low fungal burden on direct cytology, the lesion material was collected using sterile swabs and placed in Stuart transport medium (4–8°C). Seeding was performed on Sabouraud dextrose agar with chloramphenicol under aseptic conditions. Plates were incubated at 25°C for 15 days (Gremião et al., 2021; Barros et al., 2011). Presumptive identification was based on macroscopic colony characteristics and, when necessary, microscopic evaluation for septate hyphae and pyriform conidia arranged in a "daisy flower" pattern (Almeida-Paes et al., 2024).

Metabolomic Sample Processing and Derivatization

For metabolomic analysis, serum was separated by centrifugation for 10 min at 3,000 rpm, stored at −80°C, and processed for extraction within 24 h of collection. Aliquots of serum were dried in a dry block heater at 40°C until complete solvent evaporation. The dried residues were then reconstituted in 160 µL of methoxyamine hydrochloride solution (20 mg mL^{-1} in pyridine) and incubated at 65°C for 60 min for methoximation. Subsequently, 40 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was added, and the samples were incubated for an additional 60 min at

65°C to promote silylation. Derivatized samples were transferred to 200 µL inserts for subsequent GC–MS analysis. A quality control sample was prepared from pooled serum from the three clinical groups; however, the dissertation did not report QC injection frequency, analytical blanks, injection randomization, or instrumental drift assessment.

GC-MS Analysis

Chromatographic analyses were performed on a GC–MS system (Shimadzu QP2010 Ultra) equipped with an AOC-20i autosampler. Injection volume: 1 µL; split ratio 1:20; injector temperature 240°C; helium carrier gas at 1 mL/min. Separation column: Rtx-5MS (30 m × 0.25 mm × 0.25 µm). Temperature program: 80°C for 2 min, ramp 5°C/min to 300°C, hold 3 min. EI ionization (70 eV); ion source and interface at 240°C and 280°C; scan range 45–700 m/z; solvent cut time 3 min. Files were exported in mzML format, converted to ABF format (AbfConverter), and processed in MS-DIAL 4.70. Peak detection: linear-weighted moving-average smoothing (3 scans; average peak width 20 scans; minimum peak height 1000 units). Deconvolution: Sigma window 0.5, EI amplitude cutoff 10. Metabolite identification based on GCMSDB-Public-KovatsRI-VS3 and NIST 2014 libraries (spectral similarity ≥ 85%). RI calculated from alkane mixture C_7–C_{30} (Sigma-Aldrich). Peaks normalized to ribitol internal standard. All metabolite identifications correspond to putative annotation at MSI confidence levels 2–3, based on spectral library matching and retention index comparison without confirmation by authentic chemical standards.

PLS-DA Multivariate Analysis

Metabolomic data were analyzed using Python 3.11 (scikit-learn 1.3, numpy 1.24, pandas 2.0, scipy 1.11, matplotlib 3.7; complete data table and scripts provided in Supplementary Table S2 and Script S1). Data were autoscaled by mean-centering and unit-variance scaling. The PLS-DA file contains paired T0 and T60 samples from the seven infected cats that completed follow-up. Cats treated with itraconazole alone (n = 3) and those treated with itraconazole plus potassium iodide (n = 4) were analyzed together because the primary analysis compared paired T0 and T60 samples across the complete cohort. The regimen-specific groups were not designed or sized for separate multivariate analyses. Accordingly, the class label denotes the time point rather than the treatment regimen. The three healthy controls were not included in this PLS-DA. The three technical injections per animal and time point were averaged to one biological observation, resulting in seven paired observations at T0 and seven at T60. The model was fitted with two latent variables. Predictive performance was evaluated using leave-one-animal-out cross-validation, with scaling parameters estimated exclusively from the training data within each fold. Model significance was evaluated using an exact paired permutation test comprising all 128 possible within-animal exchanges of the T0 and T60 labels. The fitted sample scores on the first two latent variables are plotted in Fig. 1. The metrics reported include R^2Y and Q^2. Variable Importance in Projection scores were calculated for all metabolites, with VIP ≥ 1.0 defining the principal discriminant variables. Metabolomic inference was multivariate; no univariate per-metabolite hypothesis tests were performed.

Statistical Analysis of Clinical-Laboratory Data

Statistical analysis compared clinical and laboratory parameters between T0 and T60 in the seven infected cats (F1–F7), each serving as its own paired control. Normality of differences (T60 – T0) was evaluated using the Shapiro–Wilk test (Shapiro; Wilk, 1965). For normally distributed variables, the paired t-test was applied; otherwise, the Wilcoxon signed-rank test for paired samples was used (Conover, 1999). Significance level: $p < 0.05$ (Zar, 2010; Siegel; Castellan, 1988).

Results and Discussion

Clinical and Biochemical Parameters

Among the seven infected cats, paired comparison of T60 with T0 showed significant increases in AST, ALT, GGT, and creatinine levels and a significant reduction in hematocrit. The red blood cell and leukocyte counts and urea levels did not change significantly. Platelet counts also showed no significant paired difference ($p = 0.250$). Tables 1 and 2 summarize the clinical-laboratory data and statistical tests. Table 1 also presents descriptive data for the three healthy cats (F8–F10); however, these animals were not included in the paired inferential analysis.

Table 1
Clinical laboratory data of experimental cats at T0 and T60

Animal	RBC (×10⁶/µL)	Hct (%)	WBC (/µL)	Platelets (×10³/µL)	Urea (mg/dL)	Creat. (mg/dL)	AST (UI/L)	ALT (UI/L)	GGT (UI/L)
T0									
F1	8.2	43	14,900	250,000	24.0	0.5	43.0	26.4	1.0
F2	9.1	47	10,300	175,000	34.0	0.6	79.8	27.4	1.0
F3	9.6	45	12,100	279,000	24.0	0.6	78.0	38.2	1.0
F4	7.8	41	16,700	209,000	32.0	0.5	80.0	75.8	2.0
F5	8.3	42	11,200	300,000	22.0	0.6	14.0	78.0	2.0
F6	10.6	51	11,500	312,000	27.0	1.2	48.0	20.0	1.0
F7	9.0	42	9,800	304,000	25.0	0.7	47.0	34.0	1.0
F8*	9.4	46	6,600	221,000	38.0	1.5	55.0	79.0	4.0
F9*	9.5	43	5,700	424,000	28.0	0.8	78.8	56.0	2.0
F10*	10.3	57	6,400	155,000	38.4	1.4	58.0	32.2	2.0
T60									
F1	9.2	37	11,900	230,000	24.0	0.7	85.0	76.0	4.0
F2	7.6	44	9,100	190,000	39.0	0.9	110.6	89.2	5.0
F3	7.6	40	13,200	230,000	24.0	1.1	88.0	68.0	5.0
F4	7.5	39	11,100	200,000	32.0	0.9	85.0	95.0	3.0
F5	7.2	37	11,500	315,000	42.0	0.5	74.0	89.0	5.0
F6	8.2	49	12,200	300,000	24.0	1.5	97.0	80.0	4.0
F7	8.3	39	9,500	290,000	25.0	0.8	95.0	84.0	3.0
F8*	9.0	44	7,100	210,000	45.0	1.6	76.0	80.0	2.0
F9*	7.4	42	6,700	400,000	25.0	0.7	86.0	76.0	2.0
F10*	9.0	55	6,200	175,000	42.6	1.3	68.8	30.0	3.0

* Control group animals (F8–F10). RBC: red blood cells; Hct: hematocrit; WBC: white blood cells; Creat.: creatinine; AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase.

Statistical analysis indicated a significant elevation in AST ($p = 0.004$), ALT ($p = 0.002$), and GGT ($p < 0.001$). Hematocrit decreased significantly ($p < 0.001$), and creatinine increased significantly ($p = 0.018$). Red blood cells ($p = 0.059$), leukocytes ($p = 0.254$), platelets ($p = 0.250$), and urea ($p = 0.500$) did not change significantly. The improvement of cutaneous lesions together with increased hepatic enzyme activities indicates that clinical and biochemical responses followed distinct trajectories during treatment. Because all cats received itraconazole and four also received potassium iodide, the enzyme changes are interpreted as a treatment-period hepatic response, consistent with the reported biochemical effects of these antifungals in cats (Meyer; Harvey, 2004; Thrall et al., 2012; Gremião et al., 2021). The modest increase in creatinine occurred without a significant change in urea and is therefore reported as a concurrent biochemical change rather than evidence of renal dysfunction.

Table 2
Statistical tests were applied to the study data.

Variable	Observed change	Test used	*p*-value	Significance
RBC	Slight decrease	Paired t-test	0.059	Not significant
Hematocrit	Marked decrease	Paired t-test	< 0.001	Significant
Leukocytes	No clear pattern	Paired t-test	0.254	Not significant
AST	Marked increase	Paired t-test	0.004	Significant
ALT	Marked increase	Paired t-test	0.002	Significant
GGT	Slight increase	Paired t-test	< 0.001	Significant
Urea	Slight variation	Wilcoxon	0.500	Not significant
Creatinine	Slight increase	Paired t-test	0.018	Significant
Platelets	Slight decrease	Paired t-test	0.250	Not significant

AST: aspartate aminotransferase; ALT: alanine aminotransferase; GGT: gamma-glutamyl transferase; RBC: red blood cells

Metabolomic Analysis and Interpretation of PLS-DA Coefficients

Serum GC-MS metabolomics combined with PLS-DA distinguished the fitted T0 and T60 profiles of the seven infected cats (Fig. 1), while the corresponding coefficient and VIP plots are presented in Fig. 2. The model showed strong fit to the observed data ($R^2Y = 0.974$), while leave-one-animal-out cross-validation yielded $Q^2 = 0.943$. Exact paired permutation testing across all 128 possible within-animal label exchanges supported the distinction between time points ($p = 0.016$). To guide biological interpretation, VIP scores were calculated for all 30 metabolites. Compounds with VIP ≥ 1.0 were treated as primary discriminant variables, and the remaining compounds were considered secondary observations (Saccenti et al., 2014; Worley; Powers, 2013).

VIP analysis identified a baseline profile dominated by four long-chain aliphatic hydrocarbons: docosane (VIP = 1.608), pentacosane (VIP = 1.606), 3-ethyl-2,6,10-trimethylundecane (VIP = 1.604), and dodecane (VIP = 1.594), all of which were more abundant at T0. Alkanes have been associated with the products of non-enzymatic oxidative fragmentation of polyunsaturated fatty acids (Catalá, 2011; Niki, 2008). Their greater abundance at T0 is therefore compatible with oxidative processes during active disease, but this interpretation remains indirect because established biochemical markers of oxidative stress were not measured. The hepatic enzymes cannot be considered concurrent evidence for the T0 alkane profile because AST, ALT, and GGT increased at T60 rather than at baseline.

During the 60-day treatment period, the relative abundance of the four baseline-discriminant alkanes decreased, whereas a different set of metabolites became more prominent at T60. Tetrahydrolavandulol (VIP = 1.430) and 1-tetradecene (VIP = 1.424) showed the strongest discrimination toward T60. Their temporal variation characterizes the post-treatment profile and supports their selection as candidates for targeted biochemical investigation.

L-iditol (VIP = 1.160), myo-inositol (VIP = 1.118), L-5-oxoproline (VIP = 1.114), and stearic acid (VIP = 1.082) also exceeded the VIP ≥ 1.0 threshold and showed greater abundance at T60. Their known involvement in carbohydrate, osmotic, glutathione-related, and lipid metabolism provides a biologically relevant context for the metabolic transition observed between T0 and T60.

Two additional compounds exceeded the VIP threshold at T60. 5,6-Dihydrouracil (VIP = 1.128) was more abundant at T60; however, its biological interpretation remains uncertain because no directly applicable supporting reference was identified. Urea (VIP = 1.116) also contributed to multivariate discrimination; however, conventional serum urea did not differ significantly between T0 and T60 ($p = 0.500$). Consequently, the results do not support an inference of altered protein catabolism or renal nitrogen handling.

Chlorogenic acid (VIP = 1.111), although above the VIP threshold, is most likely of dietary origin. As a phenolic compound found in many commercial cat foods containing plant-derived ingredients (Clifford, 2000; Verbrugghe; Hesta, 2017), its variation across time points may track dietary intake rather than the metabolic response to infection or treatment; therefore, it should be interpreted with caution.

Regarding annotation confidence, all metabolite identifications in this study are putative (MSI confidence levels 2–3), derived from spectral library matching and retention index comparison without confirmation by authentic chemical standards. Structural assignments, therefore, carry inherent uncertainty, and biological interpretations should be considered exploratory and provisional until targeted analyses with reference compounds are conducted.

Seven infected cats completed the paired T0–T60 evaluation. The paired design reduces between-animal variability, but the small cohort limits the stability and generalizability of the multivariate model. Because the healthy controls were not included in the PLS-DA, the results demonstrate differences between T0 and T60 among treated infected cats but do not establish a metabolomic difference

between healthy and infected animals. In addition, itraconazole-only and combination-treatment animals were pooled, preventing attribution of the metabolic changes to a specific therapeutic regimen. The findings should therefore be interpreted as exploratory associations with the treatment period.

Secondary observations below VIP = 1.0 included 2-hydroxybutyric acid, DL-beta-hydroxybutyric acid, asparagine, simple sugars, palmitic acid, and glycerol. Because these metabolites did not reach the predefined VIP threshold and were not evaluated by univariate hypothesis tests, they were not used to support the principal biological interpretation.

Taken together, the exploratory metabolomic data show a reproducible distinction between the baseline and 60-day serum profiles of the seven treated cats. The greater abundance of selected alkanes at T0 is compatible with oxidative processes described in the literature, but oxidative stress and recovery pathways were not measured directly. The paired design supports evaluation of temporal change, whereas the small sample, pooled treatment regimens, non-standardized diets, putative metabolite annotations, and absence of healthy controls from the PLS-DA restrict causal and diagnostic interpretation. The 13 metabolites with VIP ≥ 1.0 should therefore be regarded as candidates associated with the T0–T60 treatment period rather than validated markers of infection status or therapeutic response.

Conclusions

This exploratory GC-MS metabolomics study identified differences between the baseline and 60-day serum profiles of seven cats treated for sporotrichosis. Four alkanes associated in the literature with lipid peroxidation were more abundant at T0, whereas nine other metabolites with VIP ≥ 1.0 were more abundant at T60. These patterns are compatible with temporal metabolic changes during infection and treatment, but they do not directly demonstrate systemic oxidative stress or metabolic recovery. AST, ALT, GGT, and creatinine increased and hematocrit decreased during follow-up; the study design does not distinguish treatment effects from changes related to infection or clinical evolution. The PLS-DA model ($R^2Y = 0.974$, $Q^2 = 0.943$, exact paired $p = 0.016$) identified 13 putatively annotated discriminant metabolites. These compounds are exploratory candidates associated with the T0–T60 treatment period and require validation in larger cohorts with standardized diets, separate treatment groups, authentic chemical standards, and healthy controls included in the metabolomic comparison.

Declarations

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Competing interests

The authors declare no conflicts of interest.

Author Contribution

J.O.C. and M.F.C.S. conceived and designed the study. J.O.C., G.O.C., J.V.A., I.L.B.P., and T.O.C. contributed to animal selection, clinical monitoring, sample collection, and data acquisition. G.H.S., V.G.N., and M.O.S. contributed to sample processing, instrumental analysis, and metabolomic data interpretation. J.O.C. and M.F.C.S. analyzed and interpreted the clinical and metabolomic results. M.F.C.S. supervised the study and coordinated manuscript preparation. J.O.C. and M.F.C.S. wrote the initial manuscript. All authors critically reviewed the manuscript, approved the final version, and agreed to be accountable for the work.

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Figures

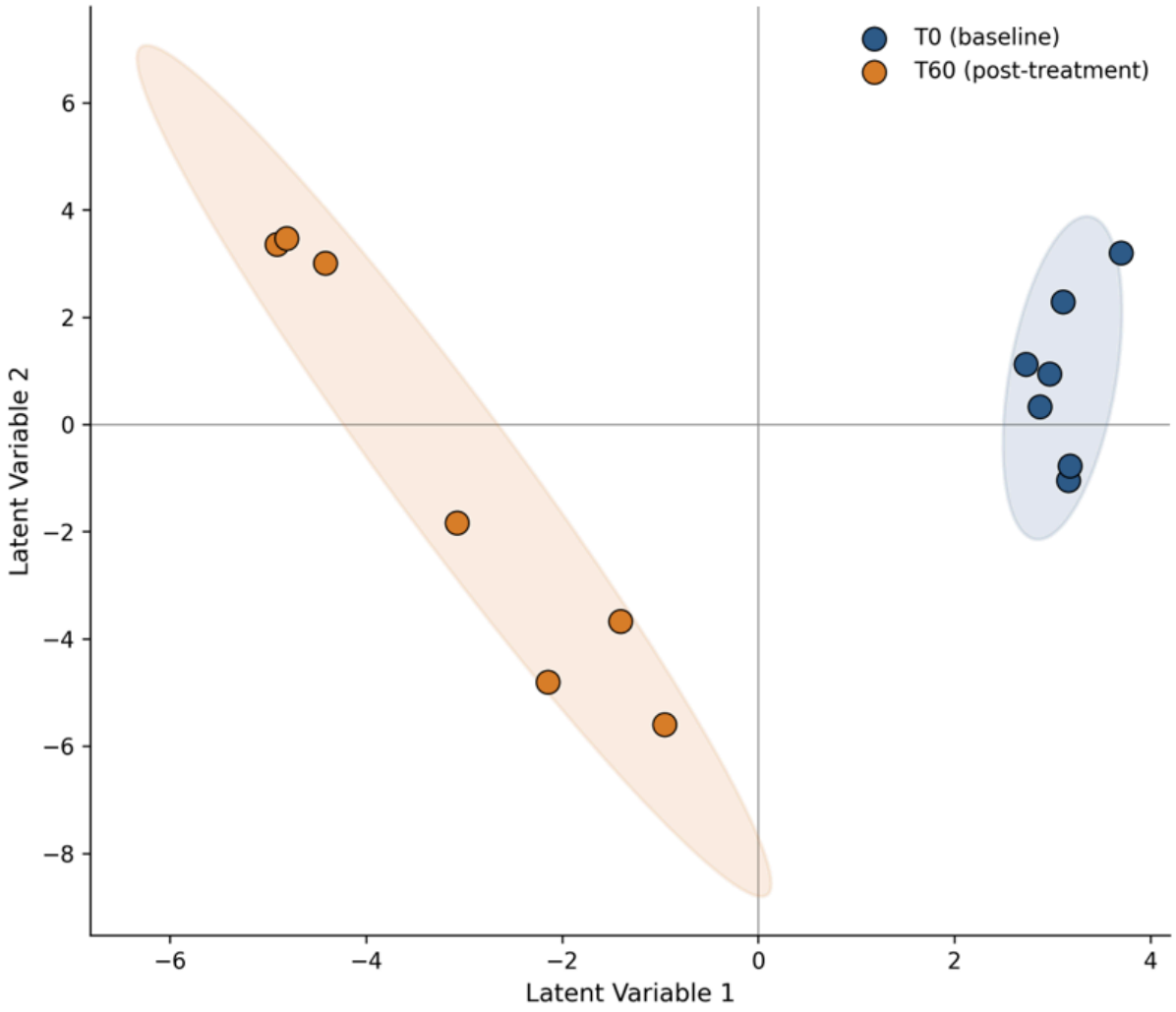


Figure 1

PLS-DA score plot for paired serum samples from seven infected cats at T0 and T60. Each circle represents one biological observation after averaging the three technical injections. The shaded regions are visual guides for the distribution of each time point. $R^2Y = 0.974$; leave-one-animal-out $Q^2 = 0.943$; exact paired permutation $p = 0.016$.

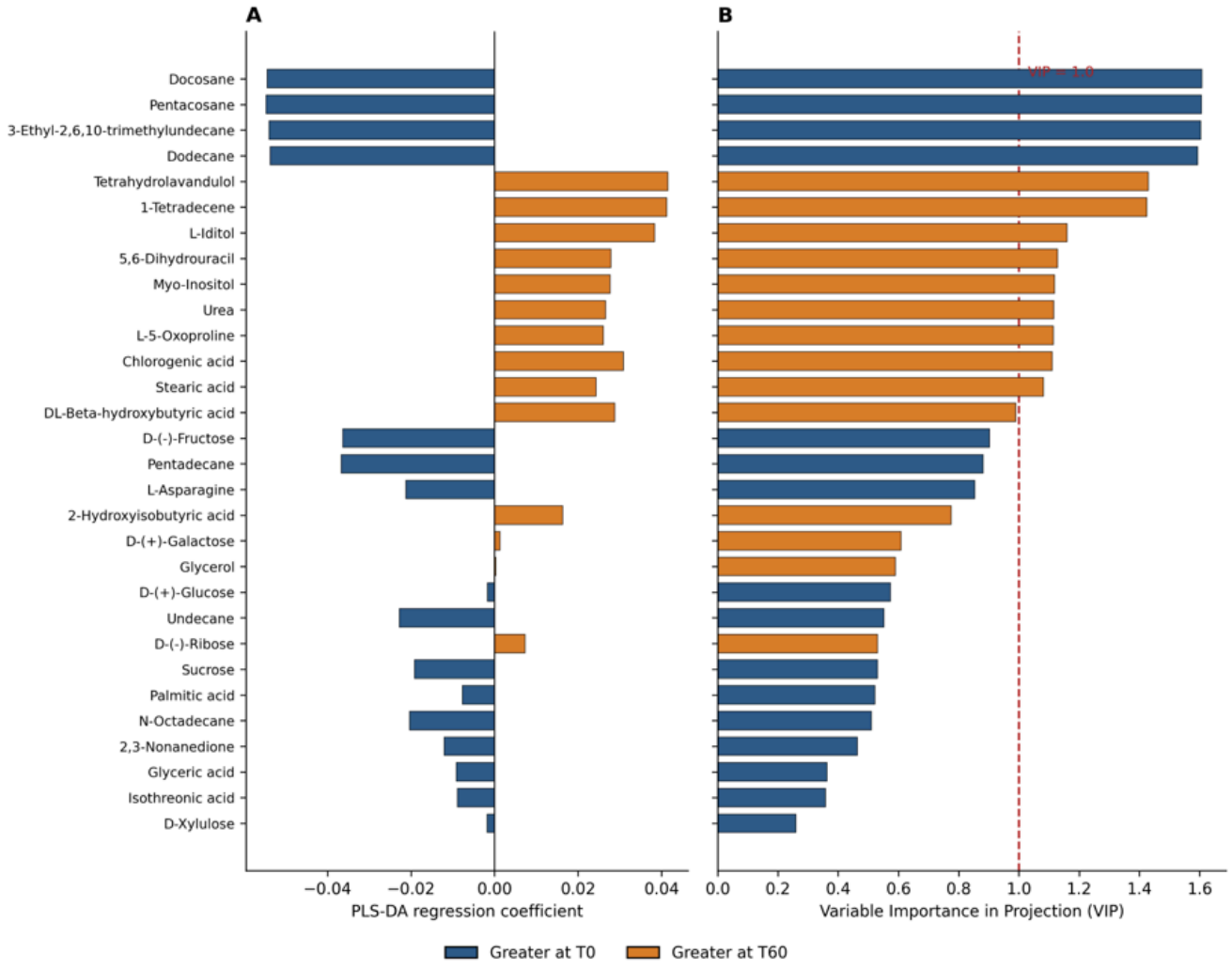


Figure 2

PLS-DA coefficient plot (A) and Variable Importance in Projection scores (B). The red dashed line in panel B indicates the VIP = 1.0 threshold. Blue bars indicate greater abundance at T0, and orange bars indicate greater abundance at T60.

Supplementary Files

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- SupplementaryMaterialSporotrichosisfinal.docx