Received: 24 June 2024 | Accepted: 4 December 2024

DOI: 10.1111/jvim.17285

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE

Small Animal Internal Medicine Hepatology

Plasma amino acid profiles of dogs with the hepatocutaneous syndrome and dogs with other chronic liver diseases

Rommaneeya Leela-arporn[1,2] | Karah Burns DeMarle[1] | Cailin R. Heinze[1] | Cynthia R. L. Webster[1]

[1]Cummings School of Veterinary Medicine at Tufts University, North Grafton, Massachusetts, USA

[2]Chulabhorn Royal Academy, Bangkok, Thailand

Correspondence
Rommaneeya Leela-arporn, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA.
Email: rommaneeya.leela-arporn@colostate.edu

Present addresses
Rommaneeya Leela-arporn, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA; Karah Burns DeMarle, MedVet Norwalk, Norwalk, Connecticut, USA; and Cailin R. Heinze, Mark Morris Institute, Topeka, Kansas, USA.

Funding information
Companion Animal Health Fund

Abstract

Background: Dogs with hepatocutaneous syndrome (HCS) have marked plasma hypoaminoacidemia, but its occurrence in dogs with chronic liver diseases not associated with HCS (non-HCS CLD) is unknown.

Objectives: To determine if plasma hypoaminoacidemia occurs in dogs with non-HCS CLD, compare plasma amino acid (PAA) profiles between dogs with non-HCS CLD and HCS, and define a sensitive and specific PAA pattern for diagnosing HCS.

Animals: Data were collected from client-owned dogs, a prospective cohort of 32 with CLD and 1 with HCS, and a retrospective cohort of 7 with HCS.

Methods: Prospective study. Dogs with chronic serum liver enzyme increases were recruited after hepatic biopsy. Plasma amino acid profiles were measured using high-performance liquid chromatography. Plasma amino acid concentrations were compared between dogs with non-HCS CLD and HCS. Regression analysis was performed to identify a unique PAA pattern for HCS diagnosis.

Results: Twelve dogs each with vacuolar hepatopathy or chronic hepatitis and 8 dogs with congenital disorders (primary hypoplasia of the portal vein or ductal plate malformations) were enrolled. Compared to non-HCS CLD dogs, HCS dogs had significantly lower plasma concentrations of several amino acids. Regression analysis revealed that glutamine, glycine, citrulline, arginine, and proline concentrations less than 30% of the mean reference value had 100% sensitivity, specificity for diagnosing HCS.

Conclusions and Clinical Importance: Generalized plasma hypoaminoacidemia does not accompany non-HCS CLD. Concentrations of 5 specific amino acids less than 30% of the mean reference value can serve as a noninvasive biomarker for diagnosing HCS.

KEYWORDS
canine, hepatic disease, hypoaminoacidemia, metabolic disease

Abbreviations: AAA, aromatic amino acid; ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCAA, branched-chain amino acid; CLD, chronic liver disease; CH, chronic hepatitis; CPSS, congenital portosystemic shunts; HCS, hepatocutaneous syndrome; LASSO, least absolute shrinkage and selection operator; non-HCS CLD, chronic liver diseases not associated with HCS; PAA, plasma amino acid; ROC, receiver operating characteristic; SSA, sulfosalicylic acid; TAA, total amino acid; VH, vacuolar hepatopathy.

1 | INTRODUCTION

Hepatocutaneous syndrome (HCS) is a metabolic disorder seen in middle-aged to older dogs that includes characteristic dermatologic and hepatic imaging changes and is always accompanied by generalized plasma hypoaminoacidemia.[1-5] The histopathology of the skin lesions is consistent with a superficial necrolytic dermatitis, on ultrasound the liver is nodular and has a honeycomb-like appearance and the plasma amino acid profile in these dogs showed a marked (>50%) depletion in many amino acids. This syndrome is associated with considerable morbidity and high mortality rates,[1-4,6-8] and early diagnosis could increase treatment options and success.

In the past, the diagnosis of HCS has been made by some combination of gross (hyperkeratotic ulcerative, crusting lesions of the paw pads, mucocutaneous junctions, and pressure points) and microscopic appearance (diffuse parakeratotic, hyperkeratosis, intracellular edema, and basal cell hyperplasia) of typical skin lesions, ultrasound appearance of the liver (typical honeycomb- or Swiss cheese-like appearance) and a presence of a degenerative vacuolar hepatopathy on hepatic biopsy.[6-13] Recent studies have suggested that the determination of plasma amino acid (PAA) profiles to detect the presence of severe plasma hypoaminoacidemia could be used as an early and noninvasive diagnostic biomarker of HCS.[1-5] Thus, the diagnosis of HCS could be made noninvasively by PAA analysis, in combination with the appearance of skin and liver lesions, avoiding the need for skin or hepatic biopsy and enabling a prompt diagnosis and initiation of therapy. Additionally, because recent data support that a hypoaminoacidemic hepatopathy can be present in the absence of skin lesions and may represent an earlier stage of the condition,[1,3,5] diagnostic options that do not require evaluation of skin changes, such as concentrations of PAA, become more important in the diagnosis of this condition.[1-5]

Although PAA concentrations in HCS have been investigated in several studies, less is known about PAA concentrations in other chronic liver diseases that might need to be differentiated from HCS. Plasma amino acid concentrations are reported in both benign nodular disease and malignant hepatocellular cancer in dogs,[14] as well as in dogs with congenital portosystemic shunts,[15] with none of these conditions showing a pattern of profound hypoaminoacidemia. Although available studies in dogs with naturally occurring chronic hepatitis have not reported consistent decreases in plasma amino acids, these studies either failed to evaluate complete PAA profiles or did not provide details on histopathological findings and/or use accepted World Small Animal Veterinary Association terminology to report these findings.[2,16-18] Most importantly, because dogs with HCS have a vacuolar hepatopathy, the PAA of dogs with non-HCS associated vacuolar hepatopathy has not been reported.

In the current study, the primary objective was to prospectively measure PAA profiles in dogs with non-HCS CLD and compare these profiles with the PAA profile from a retrospective cohort of dogs diagnosed with HCS. A secondary objective was to define a pattern of plasma hypoaminoacidemia that can be considered characteristic of HCS. We hypothesized that dogs with non-HCS CLD would not have the profound plasma hypoaminoacidemia seen in dogs with HCS and that there would be a pattern of PAA depletion with a high sensitivity and specificity for the diagnosis of HCS.

2 | MATERIALS AND METHODS

2.1 | Study design and case selection criteria

This was a prospective study conducted from 2017 to 2019 at the Foster Hospital for Small Animals at Cummings School of Veterinary Medicine at Tufts University. Dogs presenting for a history of a chronic liver disease defined as chronic increases in serum alanine aminotransferase (ALT) and/or serum alkaline phosphatase (ALP) enzyme activity >2 times the upper limit of normal for more than 2 months and for which a liver biopsy was performed, were recruited. Liver biopsy samples were obtained either by ultrasound-guided percutaneous needle biopsy or by cup biopsy at laparoscopic surgery, laparotomy, or necropsy.

Dogs were enrolled in the study if they ate commercial dog food, had bloodwork, including a complete blood count and serum biochemistry profile and an abdominal ultrasound performed within a week of obtaining the liver biopsies or necropsy samples, and if their liver histopathology report, was consistent with a diagnosis of chronic liver disease including chronic hepatitis (CH), glycogen type vacuolar hepatopathy (VH), or congenital vascular/biliary diseases such as primary hypoplasia of the portal vein or a ductal plate malformation. These diagnoses were made by board-certified veterinary pathologists using World Small Animal Veterinary Association criteria[19-23] and the ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs[24] with input from a board-certified small animal internist (Cynthia R. L. Webster) with expertise in evaluating hepatic histopathology.

Briefly, all biopsy material was stained with hematoxylin and eosin, sirius red, and rhodanine. Chronic hepatitis was defined as the presence of lymphocytic, plasmacytic, or granulomatous inflammation accompanied by hepatocyte cell death and variable amounts of fibrosis. Chronic hepatitis was subcategorized as copper-associated if a quantitative copper analysis (atomic absorption spectroscopy at Colorado State University Veterinary Diagnostic Laboratories) was >600 PPM and rhodanine staining was primarily localized to the centrilobular zone with a semiquantitative score of score >2 out of 5. Cirrhosis was diagnosed when histological signs of chronic hepatitis were accompanied by architectural distortion, fibrosis, and the presence of microscopic regenerative nodules. Portal venous hypoplasia was considered present if there was evidence of lobular atrophy, arteriolar hyperplasia, and small/absent portal vessels. Ductal plate malformation was defined histologically by the presence of malformed, proliferative bile ducts in portal tracts that were embedded in increased fibrous tissue. Vacuolar hepatopathy was diagnosed when the predominant change was the appearance of glycogen-type wispy clear cavities within hepatocytes. It was categorized as a degenerative vacuolar change if there was fibrosis accompanied by areas of centrilobular parenchymal collapse.[25]

Dogs were excluded if the liver histopathology showed the presence of neoplasia or acute hepatic injury, which included the presence of a predominant neutrophilic infiltration and/or the presence of large areas of hepatocyte necrosis in the absence of signs of chronicity such as increased fibrosis or bile duct proliferation. Diet histories were obtained and any dog receiving a home-cooked diet or supplemental protein, defined as powdered protein supplements or the consistent addition of eggs, meat, or fish to the dog's daily meals, were excluded.

To compare PAA profiles of dogs with non-HCS CLD to those seen in HCS, 7 dogs with HCS from a previous cohort study as reported elsewhere were retrospectively enrolled,[4] and 1 dog with a diagnosis of HCS in 2021 was prospectively enrolled in this study. These dogs with HCS were diagnosed based on the presence of characteristic skin lesions and histopathologic findings, increases in serum ALT or ALP enzyme activity, the ultrasound appearance of a liver with disseminated hypoechoic nodules surrounded by hyperechoic bands of tissue (honeycomb-like liver), and generalized plasma hypoaminoacidemia. All ultrasounds were done by a boarded veterinary radiologist or by a resident under the supervision of a boarded diplomate.

The Clinical Science Review Committee at the Cummings School of Veterinary Medicine at Tufts University approved the protocol, and all owners signed an informed consent before enrolling dogs in the study. For the retrospective study, all owners signed a consent to use retrospective medical record information for further studies at the time of their appointment.[4]

2.2 | Medical record data collection

Medical records of all included dogs were reviewed upon enrollment for demographic information (age, body weight, breed, and sex), diet history (commercial versus home-cooked), body condition score (based on 9 point scale),[26] muscle condition score (normal, mild, moderate, or severe muscle loss),[27] and in dogs with non-HCS-CLD the presence of skin lesions at the time of hepatic biopsy.

2.3 | PAA measurement

Two milliliters of blood were drawn into a heparin tube from each prospectively included dog during preenrollment. Blood was centrifuged, and the plasma was stored frozen at −80°C. Once liver biopsy confirmed their inclusion in the study, plasma samples for PAA profiles were shipped in batches every 2 months on dry ice to the Amino Acid Laboratory at the University of California for PAA analysis.[28] In dogs with HCS that were retrospectively enrolled plasma samples had been drawn at the time of clinical presentation, processed and frozen at −80°C, and shipped within 24 hours to the same amino acid laboratory. Measurement of the PAA concentrations was performed using the same automated high-performance liquid chromatography AA analyzer (Biochrom 30; Biochrom Ltd, Holliston, Massachusetts). The analysis included the following amino acids: alanine, arginine, α-aminobutyric acid, asparagine, aspartic acid, citrulline, cystathionine, cysteine, glutamic acid, glutamine, glycine, histidine, 3-methyl-histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, hydroxyproline, serine, taurine, threonine, tryptophan, tyrosine, and valine. The total amino acid (TAA) concentrations were calculated as the sum of the concentrations of these 27 amino acids. All PAA concentrations were reported in nmol/mL.

The BCAA/AAA ratio was calculated by summing of total branched-chain amino acids (BCAAs), which include the concentrations of leucine, valine, and isoleucine, to total aromatic amino acids (AAAs), derived from the sum of the phenylalanine and tyrosine concentrations. Although histidine and tryptophan are aromatic amino acids, these amino acids are not included in the BCAA/AAA ratio or the total AAAs in this study. This exclusion aligns with the known Fischer's ratio in human medicine,[29] as well as previous studies in veterinary medicine analyzing the BCAA/AAA ratio.[2,14-16] The rationale for this is based on the pathophysiology of liver diseases, particularly hepatic encephalopathy, where the imbalance between BCAAs (leucine, isoleucine, valine) and specific AAAs (phenylalanine, tyrosine) is most relevant.[14,16,29,30] Phenylalanine and tyrosine concentrations increase because of impaired hepatic metabolism,[14,29,30] whereas histidine and tryptophan are metabolized via different pathways and do not exhibit the same pattern of accumulation or impact on liver function.[30,31]

2.4 | Statistical analysis

Categorical variables, including body condition score, muscle condition score, sex, presence of honeycomb-like liver lesion on ultrasound, and other ultrasonographic features (liver size, echotexture, presence of nodules, and abdominal effusion) were presented as numbers and percentages. Continuous variables, including age, body weight, serum liver enzyme concentrations, albumin, total bilirubin, hepatic copper values, and PAA concentrations, were expressed as medians and ranges. Univariate analysis via the Kruskal-Wallis test was used to compare nonnormally distributed data among groups. This test was followed by the Steel-Dwass test for post hoc analysis. Bonferroni correction was applied to account for multiple comparisons of PAAs. Univariate and multivariate analyses were used to compare PAA concentration in dogs with non-HCS CLD and HCS.

The significant PAA concentrations from univariate analysis were transformed into categorical data before undergoing multivariate analysis and were expressed as changes in the percentage from the mean reference value established at the analytic laboratory.[28] This transformation was done similarly to previous review literature,[4] allowing for a comparison of the severity of hypoaminoacidemia between HCS groups and non-HCS CLD groups. The percent changes were then binned into 5 categories: <15% of normal, 15.1% to 30% of normal, 30.1% to 50% of normal, 50.1% to 75% of normal, and >75% of normal.

For the multivariate analysis, a least absolute shrinkage and selection operator (LASSO) regression analysis was performed using all significant PAAs from the univariate analysis, with Akaike Information

TABLE 1 Clinical characteristics in dogs with chronic liver disease not associated with hepatocutaneous syndrome (non-HCS CLD) and with HCS.

Variable	Chronic hepatitis (n = 12)	Vacuolar hepatopathy (n = 12)	Congenital vascular/biliary disease (n = 8)	HCS (n = 8)
Signalment				
Age—median (range)	9.5 (4-13)	10.5 (6-13)	9 (7-13)	9.5 (7-11)
Sex—n, (%)				
Male	7 (58.3)	6 (50)	2 (25)	7 (87.5)
Female	5 (41.7)	6 (50)	6 (75)	1 (12.5)
Body weight—median (range)	14.2 (5.1-42.3)	13.1 (8.5-46.4)	25.8 (5.3-40)	25.7 (13.3-51.1)
Body condition score—median (range)	5 (1-8)	6 (4-9)	5 (4-7)	5 (5-8)
Muscle condition score—n (%)				
Normal	7 (58.3)	11 (91.7)	8 (100)	3/5 (60)
Mild	3 (25)	1 (8.3)	0 (0)	1/5 (20)
Moderate	1 (8.3)	0 (0)	0 (0)	1/5 (20)
Severe	1 (8.3)	0 (0)	0 (0)	0/5 (0)

Criteria (AIC) forward selection, to look for a unique PAA pattern in dogs with HCS compared to dogs with non-HCS CLD. Least absolute shrinkage and selection operator regression is a type of regression analysis that improves model prediction accuracy by selecting the most relevant variables while minimizing the impact of others, thereby reducing overfitting.[32,33] Least absolute shrinkage and selection operator regression are increasingly being used in medical diagnosis for disease outcome prediction,[34] biomarker selection,[35] and early detection of cardiovascular diseases.[36] Receiver operating characteristic (ROC) curve analysis was then performed to determine the diagnostic accuracy of the multivariate model for diagnosing HCS.

For all statistical analyses, a value of $P < .05$ was considered statistically significant ($P < .0019$ after Bonferroni correction). Statistical analyses were performed using commercial software (JMP Pro, version 17.0.0; SAS Institute Inc, Cary, North Carolina).

3 | RESULTS

3.1 | Study sample

For the study arm of dogs with non-HCS CLD, 38 dogs underwent liver biopsies or necropsy during the study period. Six dogs were excluded because of liver histopathology consistent with hepatobiliary neoplasia, nonspecific reactive hepatopathy, and a lipidotic fibrotic inflammatory disease, which was difficult to classify. Thirty-two dogs including 12 dogs with CH, 12 dogs with VH, 8 dogs with congenital vascular/biliary diseases (5 with primary hypoplasia of the portal vein and 3 with ductal plate malformations) were prospectively enrolled. In addition, 1 dog with HCS was prospectively enrolled, whereas 7 dogs with HCS from a previous study[4] were retrospectively enrolled. Dog demographics for each group are summarized in Table 1.

The breed distribution of dogs with non-HCS CLD was as follows: 9 mixed breeds, 3 Beagles, 3 Labrador retrievers, 2 English springer spaniels, 2 Italian greyhounds, 2 poodles, 2 Shetland sheepdogs, and 1 each of cocker spaniel, Doberman pinscher, French bulldog, Cavalier King Charles spaniel, Miniature schnauzer, Ocherese, Rottweiler, Shih Tzu, and Siberian husky. Breeds of dogs with HCS included 3 Labrador retrievers and 1 each of Beagle, cocker spaniel, Nova Scotia duck tolling retriever, Pitbull, and Shetland sheepdog. There was no difference in age, body condition score or weight at the time of diagnosis among dogs with VH, CH, congenital vascular/biliary disease, and HCS (Table 1). Likewise, muscle condition score was not different among groups. In most dogs, muscle condition score was normal. There was 1 dog with a mild decrease in muscle condition score in the VH group, 1 dog each with a mild and moderate decrease in the HCS group, and 3 dogs with mild and 1 each with moderate and severe decrease in the non-HCS CLD group.

3.2 | Clinical pathology

Selected clinicopathologic variables are summarized in Table 2. On serum chemistry profile, abnormally high in serum ALP and ALT enzyme activity were the most common abnormalities. The serum ALT enzyme activity was higher in 12/12 (100%), 8/12 (66%), 7/8 (88%), and 7/8 (88%) of dogs with CH, vacuolar change, congenital biliary/vascular disease and HCS, respectively. Serum ALP enzyme activity was higher in 10/12 (83%), 12/12 (100%), 6/8 (75%), and 7/8 (88%) of dogs with CH, vacuolar change, congenital biliary/vascular disease, and HCS, respectively.

Total bilirubin concentration was normal in all dogs with VH and congenital biliary/vascular disease and was higher in 6/12 dogs (50%) with CH, and 4/8 (50%) of dogs with HCS. None of the dogs with VH or congenital biliary/vascular disease had hypoalbuminemia, but 3/12 (25%) and 4/8 (50%) of dogs with CH or HCS had low albumin, respectively. Dogs with HCS had significantly higher serum total bilirubin concentrations than dogs with VH ($P = .0005$) and congenital

TABLE 2 Selected clinical pathology in dogs with chronic liver disease not associated with hepatocutaneous syndrome (non-HCS CLD) and with HCS.

Variable	Reference range	Chronic hepatitis (n = 12)	Vacuolar hepatopathy (n = 12)	Congenital vascular/ biliary disease (n = 8)	HCS (n = 8)
Hematocrit (%)	39-55	46.5 (30-56)[ab]	49 (40-58)[b]	46 (34-52)[ab]	41 (32-43)[a]
Platelet count (10^3/μL)	180-525	337 (202-503)	422 (121-601)	221 (75-391)	345.5 (164-450)
WBC (10^3/μL)	4.9-16.9	11.3 (6.4-31.1)[a]	11 (6.2-28.4)[a]	6.3 (4.7-13.6)[a]	16.1 (6.3-25.9)[b]
Neutrophils (10^3/μL)	2.8-11.5	9.6 (4.6-26.7)[ab]	8.8 (4.8-24.7)[ab]	4.5 (3.3-9.5)[a]	14.8 (4.3-24.1)[b]
ALP (IU/L)	12-127	560 (44-2597)	1271 (212-4141)	358 (58-1697)	1690 (117-3951)
ALT (IU/L)	14-86	651.5 (124-1902)[b]	131 (55-1419)[a]	173 (45-733)[ab]	343 (73-1163)[ab]
AST (IU/L)	9-54	104 (67-466)[b]	31.5 (16-131)[a]	62 (20-960)[ab]	95.5 (7-532)[ab]
GGT (IU/L)	0-10	15 (0-80)	5 (1-183)	7 (2-48)	19.5 (3-146)
Total bilirubin (mg/dL)	0.10-0.30	0.4 (0.1-12.9)[b]	0.1 (0.1-0.2)[a]	0.2 (0.1-0.3)[ab]	0.4 (0.1-1.7)[b]
Albumin (g/dL)	2.8-4.0	3.2 (2.1-4.2)[a]	4 (3-4.5)[b]	3.5 (2.9-4.2)[a]	2.8 (2.1-3.4)[a]
Glucose (mg/dL)	67-135	98 (81-154)	101 (72-130)	101.5 (82-113)	171 (87-432)

Note: [a,b]Values within a row that do not share a common superscript letter are significantly different at $P < .05$. Data are presented as median (range).
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; WBC, white blood cell count.

biliary/vascular disease ($P = .046$) and lower serum albumin than dogs with VH ($P = .0006$) and congenital biliary/vascular disease ($P = .015$). There was no significant difference in total bilirubin and serum albumin levels between dogs with HCS and CH.

Blood ammonia was higher (2.7 and 1.6 times the ULN) in the 2 dogs, in which it was determined. In addition, 2 dogs had increases in pre- and postprandial total serum bile acids from 8.3 to 38 μM and 271 to 503 μM.

3.3 | Abdominal ultrasonographic findings

All dogs had an abdominal ultrasound performed by a board-certified veterinary radiologist or by a resident under supervision by a boarded diplomate. On ultrasonographic exam, none of the non-HCS CLD dogs had changes that resembled a honeycomb-like liver, whereas the liver of all the dogs with HCS had this appearance as it was a criterion for inclusion. The ultrasound appearance was highly variable among the other 3 groups. In dogs with VH, the liver was most often enlarged (10/12, 83%) and hyperechoic (8/12, 66%) with 5/12 (42%) having 1 or more nodules. In most dogs with CH, the liver was described as having a heterogenous echotexture (7/12, 58%). Four of 12 dogs (33.3%) with CH had nodules and 2/12 (17%) had ascites; however, no acquired portosystemic shunts were identified. Half of the dogs with congenital biliary/vascular disease had a normal hepatic ultrasound and the other half had a heterogenous liver, with 2 dogs having nodules (25%), and 1 dog with ascites (12.5%).

Hepatic biopsies were obtained by ultrasound-guided percutaneous needle biopsy (14/40, 35%), laparoscopic liver biopsy (16/40, 40%), laparotomy (6/40, 15%), and necropsy (4/40, 10%). All ultrasound-guided biopsies were 16 gauge except in 1 dog with HCS where 18 gauge biopsies were obtained. Six of these 14 percutaneous biopsies (43%) were from the dogs with HCS, the 1 group, for which hepatic biopsy was not required. These dogs with HCS were in the retrospective arm of the study where the diagnosis of HCS was established with skin histopathology and plasma amino acid analysis.[4] For the other 8 percutaneous 16 gauge biopsies either 3 (4 dogs) or 4 (4 dogs) samples were obtained. Dogs in the CH category had cirrhosis (3/12, 25%), chronic hepatitis (5/12, 42%), or copper-associated chronic hepatitis (4/12, 33%). In the dogs with VH, 9/12 (75%) had diffuse vacuolar change, and 3/12 (25%) had zonal change. Underlying disorders associated with the VH were hyperadrenocorticism (5/12, 42%), gallbladder mucocele (3/12, 25%), and splanchnic neoplasia (2/12, 17%), including renal carcinoma and cecal gastrointestinal stromal tumor. In 2 dogs no underlying cause was identified. The morphologic diagnosis in the dogs in the congenital biliary/vascular disease was ductal plate malformation (5/8, 63%) and primary hypoplasia of the portal vein (3/8, 37%).

A quantitative hepatic copper was determined in 9/12 CH, 9/12 vacuolar, 8/8 congenital biliary/vascular disease, and 2/8 HCS biopsies. The median values were 819 ppm (range 78-2370 ppm), 253 ppm (range 104-497 ppm), 229 (range 113-644 ppm), and 444 (range 350-538 ppm) in CH, vacuolar, congenital biliary/vascular disease and HCS, respectively. Hepatic copper values in dogs with CH were significantly greater than those with VH or congenital biliary/vascular disease ($P = .022$). There was no significant difference in hepatic copper values in dogs with HCS compared to dogs with non-HCS. Five dogs in the CH group were diagnosed with copper-associated hepatitis.

3.4 | PAA concentrations

Comparisons of the PAA concentrations among the dogs with CH, VH, congenital vascular/biliary disease, and HCS, and between all dogs with non-HCS CLD and HCS were done using univariate and multivariate analyses. The results of univariate analysis of the PAA

TABLE 3 Plasma amino acid concentrations in dogs with chronic liver disease not associated with hepatocutaneous syndrome (non-HCS CLD) and with HCS.[A]

Amino acid (nmol/mL)	Chronic hepatitis (n = 12)	Vacuolar hepatopathy (n = 12)	Congenital vascular/biliary disease (n = 8)	HCS (n = 8)
Alanine (RR: 380-398)	469.3 (154.8-738.5)[b] 120%	489.2 (218.6-1256.6)[b] 139%	379.5 (231.2-552.7)[b] 99%	151.5 (57-294)[a] 40%
Arginine (RR: 99-105)	98 (43-161.6)[b] 97%	89.2 (46.5-180.3)[b] 100%	98.5 (37.2-165.1)[b] 96%	16 (6-30)[a] 17%
α-Aminobutyric acid (RR: 4-8)	31.5 (13.8-58.3) 551%	34.1 (18-72.1) 585%	24.7 (12.8-37.3) 405%	22.5 (8-70) 488%
Asparagine (RR: 40-42)	66.3 (42.7-113.7)[b] 172%	65.6 (34.3-97.4)[b] 150%	55.9 (30.1-89.3)[b] 130%	20 (6-34)[a] 44%
Aspartic acid (RR: 6.8-7.2)	9.7 (5.4-20.9)[b] 150%	7 (5.7-15.2)[b] 118%	7.6 (4-60.6)[b] 192%	4 (2-5)[a] 52%
Citrulline (RR: 39-43)	46.1 (26.8-126)[b] 147%	52.7 (22.8-134.2)[b] 146%	51.4 (26.3-90)[b] 128%	10.5 (6-17)[a] 27%
Cystathionine (RR: 2-4)	4.1 (1.4-15.4) 187%	9.6 (1.8-13.6) 275%	5.65 (2-13.3) 257%	3.5 (0-7) 113%
Cysteine (RR: 45-47)	8.2 (1.7-29.9) 27%	7 (0-30.4) 25%	10.95 (1.9-15.9) 22%	5 (0-7) 9%
Glutamic acid (RR: 23-25)	58.5 (29.7-95.2)[ab] 249%	46.3 (22.8-79)[ab] 188%	44.3 (21.9-83)[ab] 197%	27 (16-35)[a] 110%
Glutamine (RR: 486-504)	788 (432.9-1004)[b] 155%	757.1 (420.8-1084.2)[b] 150%	896.2 (584-1247)[b] 185%	144 (57-237)[a] 29%
Glycine (RR: 258-274)	199.4 (119.5-385.9)[b] 78%	169.5 (104.2-276.4)[b] 66%	191.9 (102.4-257)[b] 69%	75 (53-114)[a] 29%
Histidine (RR: 69-73)	88.9 (69.5-118.9)[b] 129%	84.5 (54-99)[b] 114%	82.2 (76.1-116.1)[b] 125%	54 (32-80)[a] 77%
3-Methyl-histidine (RR: 5-7)	13.4 (7.6-23.7) 242%	14 (4.2-100.8) 400%	10.3 (6.2-15.7) 186%	5.5 (0-86) 271%
Isoleucine (RR: 50-52)	71.1 (35-90.9) 135%	71.7 (49.3-139.4) 149%	51.8 (27.9-68.1) 103%	63.5 (33-150) 142%
Leucine (RR: 117-123)	140.9 (55.1-183.7) 112%	139.8 (88.7-253.6) 122%	109.9 (70.8-154.1) 93%	108.5 (76-378) 124%
Lysine (RR: 126-136)	165.8 (88.6-324.1)[b] 146%	200.8 (94.5-458.6)[b] 164%	220 (107-261.2)[b] 149%	84 (54-121)[a] 65%
Methionine (RR: 55-59)	75.5 (41-110)[b] 131%	60.3 (40.4-116.5)[b] 113%	56.2 (32.1-72.2)[b] 97%	23.5 (15-46)[a] 46%
Ornithine (RR: 33-37)	21.2 (8.5-81.9)[ab] 75%	15.6 (4.3-21.7)[ab] 45%	16 (7-38.5)[ab] 54%	5.5 (0-17)[a] 20%
Phenylalanine (RR: 44-46)	94.2 (61.2-192.2) 233%	79.1 (52.2-102) 172%	79.4 (50.8-161) 204%	84.5 (61-96) 179%
Proline (RR: 241-257)	138.4 (42.3-245.3)[b] 57%	118.7 (72.6-258.5)[b] 57%	147.3 (74.7-160.1)[b] 51%	37 (17-58)[a] 14%
Hydroxyproline (RR: 63-71)	6.6 (1.6-29) 14%	7.4 (0-26) 14%	12.8 (1.4-30.6) 21%	0 (0-17) 5%
Serine (RR: 104-110)	157.5 (72.3-341.1)[b] 149%	107.7 (48.1-160.9)[b] 102%	108.7 (60.6-154.8)[b] 103%	62.5 (27-73)[a] 53%
Taurine (RR: 75-79)	151 (26.1-256.9) 202%	118.7 (86.2-368.9) 199%	115.6 (40-144) 140%	91 (17-171) 115%
Threonine (RR: 173-183)	179.9 (122.6-572.2)[b] 128%	183 (109.6-397.7)[b] 114%	150.2 (67.6-347)[b] 100%	38 (23-54)[a] 20%
Tryptophan (RR: 58-62)	57.7 (45.5-129.4) 114%	89.1 (44.5-164) 150%	79.9 (63.7-119.8) 144%	48 (26-147) 109%
Tyrosine	46.1 (23.5-144.7)	47.1 (29.5-67.6)	50.9 (36.2-93.7)	31 (18-47)

TABLE 3 (Continued)

Amino acid (nmol/mL)	Chronic hepatitis (n = 12)	Vacuolar hepatopathy (n = 12)	Congenital vascular/biliary disease (n = 8)	HCS (n = 8)
(RR: 38-40)	158%	118%	147%	80%
Valine (RR: 154-162)	221.9 (99-264.6) 125%	203 (160.4-397.8) 139%	161.5 (84.3-217.2) 100%	166 (100-403) 125%

Note: [a,b]Values within a row that do not share a common superscript letter are significantly different at $P < .0019$ after Bonferroni correction. Data are presented as median (range) and percentage of normal.
Abbreviation: RR, reference range.
[A]Changes in the percentage from the mean reference value established at the analytic laboratory.[21] The percent changes were divided into 5 categories: <15% of normal, 15.1% to 30% of normal, 30.1% to 50% of normal, 50.1% to 75% of normal, and >75% of normal.

TABLE 4 Plasma total amino acid, branched-chain amino acid, and aromatic amino acid concentrations and calculation BCAA/AAA ratio in dogs with chronic liver disease not associated with hepatocutaneous syndrome (non-HCS CLD) and with HCS.

Amino acid (nmol/mL)	Chronic hepatitis (n = 12)	Vacuolar hepatopathy (n = 12)	Congenital vascular/biliary disease (n = 8)	HCS (n = 8)
TAAs[A]	3662.5 (2443-4604)[b]	3555 (2059-5595)[b]	3472.5 (2246-4010)[b]	1419 (1014-2206)[a]
BCAAs[B]	426.5 (189-539)	415 (298-791)	325 (183-439)	327 (209-931)
AAAs[C]	143 (96-303)	123 (92-150)	126.5 (87-255)	109 (79-141)
BCAA/AAA	2.8 (0.6-4.1)	3.6 (2.2-5.8)	2.9 (0.7-3.5)	2.9 (1.9-6.6)

Note: [a,b]Values within a row that do not share a common superscript letter are significantly different at $P < .05$. Data are presented as median (range).
Abbreviations: AAAs, aromatic amino acids; BCAAs, branched-chain amino acids; TAAs, total amino acids.
[A]TAAs were calculated as the sum of concentrations for 27 amino acids, including alanine, arginine, α-aminobutyric acid, asparagine, aspartic acid, citrulline, cystathionine, cysteine, glutamic acid, glutamine, glycine, histidine, 3-methyl-histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, hydroxyproline, serine, taurine, threonine, tryptophan, tyrosine, and valine.
[B]BCAAs were calculated as the sum of concentrations for leucine, valine, and isoleucine.
[C]AAAs were calculated as the sum of concentrations for phenylalanine and tyrosine.

profiles, the concentration of total amino acids, branched-chain amino acids, aromatic amino acids, and the calculation of the BCAA/AAA ratio in the 4 groups of dogs are summarized in Tables 3 and 4. The BCAA/AAA in 3/12 dogs in the CH category with cirrhosis were 0.62, 1.68, and 2.18, respectively.

In the univariate analysis, the concentrations of 13 individual amino acids including aspartic acid, threonine, serine, asparagine, glutamine, glycine, alanine, citrulline, methionine, lysine, histidine, arginine, and proline were significantly decreased in dogs with HCS compared to the 3 groups of dogs with non-HCS CLD (Table 3 and Data S1). The total amino acid concentrations were significantly decreased in dogs with HCS compared to the 3 groups of dogs with non-HCS CLD (Table 4). The concentrations of branched-chain amino acids, aromatic amino acids, and the calculated BCAA/AAA ratio were not significantly different among the 4 groups of dogs as shown in Table 4.

No dog with non-HCS CLD had the pattern of marked hypoaminoacidemia as seen in dogs with HCS. There were only 2 amino acids that were markedly depleted (<30% of the mean reference value) in this group of dogs. These were cysteine and hydroxyproline, as shown in Table 3. In dogs with CH, the concentrations of proline (57%), ornithine (75%), and glycine (78%) were mildly decreased from the normal reference values. In dogs with VH, the concentrations of ornithine (45%), proline (57%), and glycine (66%) were mild to moderately decreased from values in the normal reference values. In dogs with congenital biliary/vascular disease the concentrations of ornithine (54%), proline (51%), and glycine (69%) were moderately decreased from that seen in the normal reference population.

All significant PAAs from the univariate analysis were grouped into categories based on the percent change from the reference value for normal dogs[28] and were included in the LASSO regression analysis. The multivariate analysis revealed that glutamine ($P = .0009$), glycine ($P < .0001$), citrulline ($P < .0001$), arginine ($P < .0001$), and proline ($P < .0001$) concentrations less than 30% of the mean reference value were found to distinguish dogs with HCS as shown in Figure 1. Receiver operating characteristic analysis showed that the diagnostic performance of this PAA pattern for HCS represented 100% sensitivity, specificity, PPV, and NPV, with an area under the curve of 1.00.

4 | DISCUSSION

The current study found that dogs with non-HCS CLD including CH, VH, and congenital biliary/vascular disease do not have the profound plasma hypoaminoacidemia characteristically seen in dogs with HCS. Dogs with HCS had a pattern of 5 markedly decreased (<30% of the mean reference value) plasma amino acids, whereas dogs with non-HCS CLD only had mild (a percentage change from the mean reference value of 75%-100%) to moderate (a percentage change between

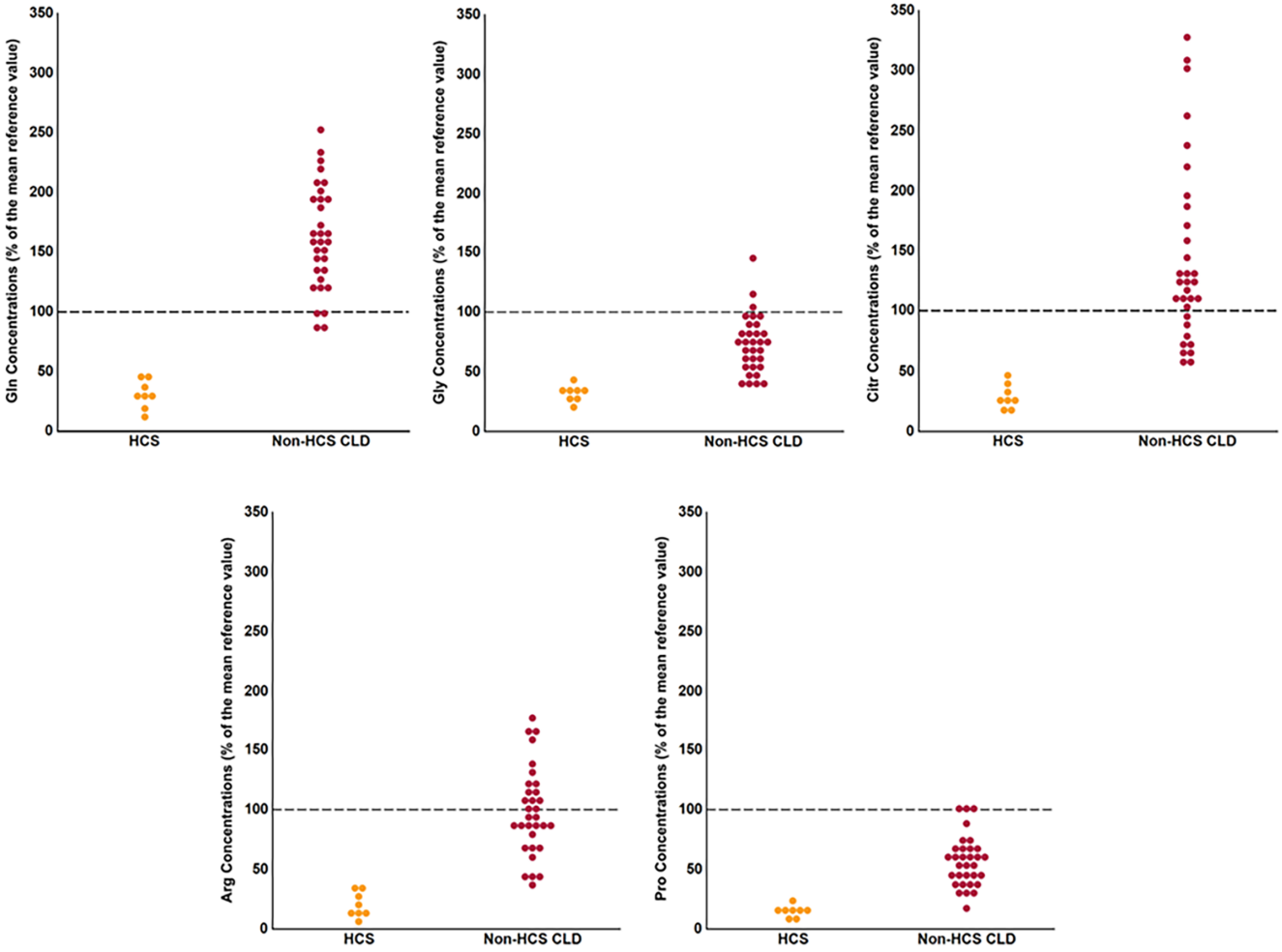

FIGURE 1 Plasma concentrations of glutamine, glycine, citrulline, arginine, and proline in dogs with hepatocutaneous syndrome (HCS) and other chronic liver diseases (non-HCS CLD) as a percentage of the mean reference value for normal dogs. The dashed horizontal lines represent the plasma amino acid concentrations for normal dogs. Glutamine ($P = .0009$), glycine ($P < .0001$), citrulline ($P < .0001$), arginine ($P < .0001$), and proline ($P < .0001$) concentrations were markedly decreased in dogs with HCS compared to dogs with non-HCS CLD. Arg, arginine; Citr, citrulline; Gln, glutamine; Gly, glycine; Pro, proline.

50% and 75% of the mean reference value) decreases in at most 3 amino acids. We found that marked depletions (<30% of the reference values) in glutamine, glycine, arginine, citrulline, and proline concentrations were consistently seen in dogs with HCS and not in dogs with non-HCS CLD. This pattern of marked decreases in these 5 plasma amino acids had a high sensitivity and specificity for the diagnosis of HCS.

Similar to other studies,[2-5] we found generalized hypoaminoacidemia in dogs with HCS with total plasma amino acids decreased by approximately 60% and significant decreases in 13 individual amino acids. In contrast, total amino acid concentrations in dogs with non-HCS CLD were not decreased over reported values in normal dogs.[2,16,17] Only 2 amino acids showed marked decreases (<30% of the normal reference value) in both the non-HCS CLD and HCS dogs which were cysteine and hydroxyproline. Decreases in cysteine levels could be attributed to the absence of deproteinization using sulfosalicylic acid (SSA) in the sample preparation process for PAA analysis,[37] which is 1 of the limitations of this study. Decreases in hydroxyproline have previously been reported in dogs with chronic hepatitis and CPSS[16] although not in dogs with hepatocellular carcinoma or gallbladder mucocele. Hydroxyproline is a nonproteinogenic amino acid associated with fibrosis and collagen metabolism. The reason for its profound depletion in a broad range of fibrotic and nonfibrotic hepatic disorders in this and other studies is not readily apparent.

A novel finding of this study is that a characteristic pattern of marked decreases in 5 plasma amino acids, including glutamine, glycine, arginine, citrulline, and proline, had a high sensitivity and specificity for the diagnosis for the presence of HCS. This finding suggests that this amino acid pattern may be used as a biomarker for the disease. Data from a retrospective evaluation of PAA in HCS corroborates that these specific 5 amino acids are consistently depleted in HCS.[3,5,9,11] In most, but not all, reports this decrease is moderate to marked. These same 5 amino acids were not depleted in the non-HCS CLD investigated in this study and this pattern has not been reported in dogs with malignant or benign hepatic tumors, gallbladder mucocele, extrahepatic congenital portosystemic shunts or acute hepatopathies.[2,14-18,38-40] Further corroborating the specificity of this 5-amino acid pattern is the

finding that studies examining PAA profiles in dogs with nonhepatic diseases including protein-losing enteropathy, protein-losing nephropathy, mammary gland tumors, brain tumors, and critical illness have not found this pattern.[41-47] Although the exact pathophysiology underlying this profound decrease in amino acids in dogs with HCS remains unclear, it is possible that HCS might be linked to dysregulated protein metabolism caused by metabolic or hormonal dysfunction. Therefore, these combined mechanisms likely contribute to the clinical features of HCS, such as the characteristic cutaneous lesions and the characteristic vacuolar hepatopathy results in the honeycomb-like appearance of the liver on ultrasound.[1-13,48-50]

Reference laboratories typically report plasma amino acid panels as a bank of 18 to 27 individual amino acids. Identification of marked decreases in 5 amino acids (glutamine, glycine, citrulline, proline, and arginine) can serve as a biomarker that simplifies the evaluation of this data to arrive at the diagnosis of HCS. Accumulating evidence also suggests that plasma hypoaminoacidemia can precede the development of skin and hepatic lesions.[1-5] Thus, PAA could permit a prompt noninvasive method to identify this metabolic disorder and permit early intervention to potentially prevent the development of painful and potentially life-threatening skin lesions.

A recent study of dogs with HCS suggested the plasma 1-methyl-histidine values <7 nmol/mL and plasma cystathionine <7.5 nmol/mL were robust biomarkers for HCS with sensitivities and specificities of 95% and 100% and 85% and 100%, respectively.[5] In the current study, however, depletion of cystathionine was noted in only 4/8 dogs with HCS. Too few dogs had 1-methyl-histidine measured to draw any conclusion. The reason for this discrepancy in cystathionine concentration is not readily apparent. Both studies used the same reference laboratory for PAA analysis but perhaps differences in sample handling or the study sample might explain the differences seen.

This study had several limitations. First, the number of dogs enrolled in each group in this study was small and the use of the percentage of the mean reference value might not be the ideal methodology for assessing the severity of hypoaminoacidemia. Thus, the calculation of diagnostic accuracy is limited. Second, plasma samples used to analyze PAAs in this study were not deproteinized with SSA; thus, cysteine concentrations could not be determined.[37] Third, several board-certified pathologists were involved in the histopathologic interpretation of the hepatic biopsies which could have led to discrepancies in the classification of the hepatic disease; however, this seems unlikely as all biopsies were also evaluated by 1 of the authors who has expertise in hepatobiliary disease. Fourth, there was no control group of dogs in the study; the reference values from normal dogs assayed[28] at the same diagnostic laboratory were used as a basis for comparison. The dogs with the non-HCS CH were not age- and breed-matched to the retrospective cases of HCS. Next, the dogs in the study ate a variety of different diets and likely varied in their intake of both total protein and specific amino acids and this information wasn't readily quantifiable. However, the reference values for PAA also included dogs eating a large variety of commercial diets. Urinary amino acid profiles were not analyzed in this study, which could be considered in future research once reference values in normal dogs have been established. Another limitation was that feed was not withheld from dogs for a consistent time before blood collection, although the reference laboratory indicated that withholding food was not necessary.[51] It should also be noted that in 6 cases quantitative hepatic copper analysis was determined on a single 16 gauge needle biopsy specimen which might have yielded less than the optimum 20 mg of tissue necessary to obtain the most accurate hepatic copper quantification with atomic absorption methods. Lastly, the dogs were also on a variety of different medications; however, these medications were prescribed to treat each dog's underlying disease and would not be expected to alter protein metabolism.

In conclusion, the results of this study suggest that overall plasma hypoaminoacidemia does not occur in dogs with non-HCS CLD, including chronic hepatitis, vacuolar hepatopathy, or congenital vascular/biliary disease. Marked depletions (<30% of the normal reference range) in arginine, citrulline, glutamine, glycine, and proline concentration were a characteristic PAA pattern seen in dogs with HCS. Thus, the PAA analysis could be considered a simple, safe, and potentially promising noninvasive biomarker for the diagnosis of HCS in dogs.

ACKNOWLEDGMENT

Funding provided by Companion Animal Health Fund at Cummings School of Veterinary Medicine at Tufts University.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Clinical Science Review Committee at the Cummings School of Veterinary Medicine at Tufts University.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Rommaneeya Leela-arporn https://orcid.org/0000-0001-9933-135X
Cailin R. Heinze https://orcid.org/0000-0002-8524-6346
Cynthia R. L. Webster https://orcid.org/0000-0003-3151-1826

REFERENCES

1. Hall-Fonte DL, Center SA, McDonough SP, et al. Hepatocutaneous syndrome in Shih tzus: 31 cases (1996-2014). *J Am Vet Med Assoc*. 2016;248:802-813.
2. Outerbridge CA, Marks SL, Rogers QR. Plasma amino acid concentrations in 36 dogs with histologically confirmed superficial necrolytic dermatitis. *Vet Dermatol*. 2002;13:177-186.
3. Loftus JP, Center SA, Lucy JM, et al. Characterization of aminoaciduria and hypoaminoacidemia in dogs with hepatocutaneous syndrome. *Am J Vet Res*. 2017;78:735-744.
4. DeMarle KB, Webster CRL, Penninck D, et al. Approach to the diagnosis of hepatocutaneous syndrome in dogs: a retrospective study and literature review. *J Am Anim Hosp Assoc*. 2021;57:15-25.

5. Loftus JP, Center SA, Astor M, Miller AJ, Peters-Kennedy J. Clinical features and amino acid profiles of dogs with hepatocutaneous syndrome or hepatocutaneous-associated hepatopathy. *J Vet Intern Med*. 2022;36:97-105.
6. Bach JF, Glasser SA. A case of necrolytic migratory erythema managed for 24 months with intravenous amino acid and lipid infusions. *Can Vet J*. 2013;54:873-875.
7. Hill PB, Auxilia ST, Munro E, Genovese L, Silkstone MA, Kirby B. Resolution of skin lesions and long-term survival in a dog with superficial necrolytic dermatitis and liver cirrhosis. *J Small Anim Pract*. 2000;41:519-523.
8. Jacobson LS, Kirberger RM, Nesbit JW. Hepatic ultrasonography and pathological findings in dogs with hepatocutaneous syndrome: new concepts. *J Vet Intern Med*. 1995;9:399-404.
9. Gross TL, Song MD, Havel PJ, Ihrke PJ. Superficial necrolytic dermatitis (necrolytic migratory erythema) in dogs. *Vet Pathol*. 1993;30:75-81.
10. Miller WHJ, Anderson WI, McCann JP. Necrolytic migratory erythema in a dog with a glucagon-secreting endocrine tumor. *Vet Dermatol*. 1991;2:179-182.
11. Nam A, Han SM, Go DM, Kim DY, Seo KW, Youn HY. Long-term management with adipose tissue-derived mesenchymal stem cells and conventional treatment in a dog with hepatocutaneous syndrome. *J Vet Intern Med*. 2017;31:1514-1519.
12. Nyland TG, Barthez PY, Ortega TM, Davis CR. Hepatic ultrasonographic and pathologic findings in dogs with canine superficial necrolytic dermatitis. *Vet Radiol Ultrasound*. 1996;37:200-205.
13. March PA, Hillier A, Weisbrode SE, et al. Superficial necrolytic dermatitis in 11 dogs with a history of phenobarbital administration (1995-2002). *J Vet Intern Med*. 2004;18:65-74.
14. Devriendt N, Paepe D, Serrano G, et al. Plasma amino acid profiles in dogs with closed extrahepatic portosystemic shunts are only partially improved 3 months after successful gradual attenuation. *J Vet Intern Med*. 2021;35:1347-1354.
15. Leela-arporn R, Ohta H, Tamura M, et al. Plasma-free amino acid profiles in dogs with hepatocellular carcinoma. *J Vet Intern Med*. 2019;33:1653-1659.
16. Lawrence YA, Bishop MA, Honneffer JB, et al. Untargeted metabolomic profiling of serum from dogs with chronic hepatic disease. *J Vet Intern Med*. 2019;33:1344-1352.
17. Imbery CA, Dieterle F, Ottka C, et al. Metabolomic serum abnormalities in dogs with hepatopathies. *Sci Rep*. 2022;12:5329. doi:10.1038/s41598-022-09056-5
18. Habermaass V, Gori E, Abramo F, et al. Serum amino acids imbalance in canine chronic hepatitis: results in 16 dogs. *Vet Sci*. 2022;9:455. doi:10.3390/vetsci9090455
19. Cullen JM, Fieten H, Grinwis GCM, et al. Morphological classification of circulatory disorders of the canine and feline liver. In: van den Ingh T, ed. *WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases (2006)* [Updated Electronic Version; Chapter 4]. Society of Comparative Hepatology; 2021. Accessed September 23, 2024. https://comparativehepatology.org/wp-content/uploads/2021/09/chapter_4.pdf
20. van den Ingh TSGAM, Cullen JM, Fieten H, et al. Morphological classification of biliary disorders of the canine and feline liver. In: van den Ingh T, ed. *WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases (2006)*, [Updated Electronic Version; Chapter 5]. Society of Comparative Hepatology; 2021. Accessed September 23, 2024. https://comparativehepatology.org/wp-content/uploads/2021/09/chapter_5.pdf
21. Grinwis GCM, Cullen JM, Fieten H, et al. Morphological classification of parenchymal disorders of the canine and feline liver—1. Normal histology, reversible hepatocytic injury and hepatic amyloidosis. In: van den Ingh T, ed. *WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases (2006)*, [Updated Electronic Version; Chapter 6]. Society of Comparative Hepatology; 2021. Accessed September 23, 2024. https://comparativehepatology.org/wp-content/uploads/2021/09/chapter_6.pdf
22. van den Ingh TSGAM, Cullen JM, Grinwis GCM, et al. Morphological classification of parenchymal disorders of canine and feline liver—2. Hepatocellular death, hepatitis and cirrhosis. In: van den Ingh T, ed. *WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases (2006)*, [Updated Electronic Version; Chapter 7]. Society of Comparative Hepatology; 2021. Accessed September 23, 2024. https://comparativehepatology.org/wp-content/uploads/2021/09/chapter_7.pdf
23. Cullen JM, van den Ingh TSGAM, Grinwis GCM, et al. Morphological classification of parenchymal disorders of canine and feline liver—3. Hepatic abscesses and granulomas, hepatic metabolic storage disorders and miscellaneous conditions. In: van den Ingh T, ed. *WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases (2006)*. [Updated Electronic Version; Chapter 8]. Society of Comparative Hepatology; 2021. Accessed September 23, 2024. https://comparativehepatology.org/wp-content/uploads/2021/09/chapter_8.pdf
24. Webster CRL, Center SA, Cullen JM, et al. ACVIM consensus statement on the diagnosis and treatment of chronic hepatitis in dogs. *J Vet Intern Med*. 2019;33:1173-1200.
25. Sepesy LM, Center SA, Randolph JF, Warner KL, Erb HN. Vacuolar hepatopathy in dogs: 336 cases (1993-2005). *J Am Vet Med Assoc*. 2006;229:246-252.
26. Laflamme D. Development and validation of a body condition score system for dogs. *Canine Pract*. 1997;22:10-15.
27. Freeman LM, Michel KE, Zanghi BM, Vester Boler BM, Fages J. Evaluation of the use of muscle condition score and ultrasonographic measurements for assessment of muscle mass in dogs. *Am J Vet Res*. 2019;80:595-600.
28. Delaney SJ, Kass PH, Rogers QR, Fascetti AJ. Plasma and whole blood taurine in normal dogs varying size fed commercially prepared food. *J Anim Physiol Anim Nutr (Berl)*. 2003;87:236-244.
29. Soeters PB, Fischer JE. Insulin, glucagon, amino acid imbalance, and hepatic encephalopathy. *Lancet*. 1976;2:880-882.
30. Dejong CHC, van de Poll MCG, Soeters PB, Jalan R, Olde Damink SWM. Aromatic amino acid metabolism during liver failure. *J Nutr*. 2007;137:1579S-1585S.
31. Holeček M. Histidine in health and disease: metabolism, physiological importance, and use as a supplement. *Nutrients*. 2020;12:848. doi:10.3390/nu12030848
32. Tibshirani R. Regression shrinkage and selection via the LASSO. *J Royal Stat Soc*. 1996;58:267-288.
33. Zou H, Hastie T. Regularization and variable selection via the elastic net. *J Royal Stat Soc*. 2005;67:301-320.
34. Chintalapudi N, Angeloni U, Battineni G, et al. LASSO regression modeling on prediction of medical terms among seafarers' health documents using tidy text mining. *Bioengineering*. 2022;9:124. doi:10.3390/bioengineering9030124
35. Ternès N, Rotolo F, Michiels S. Empirical extensions of the LASSO penalty to reduce the false discovery rate in high-dimensional Cox regression models. *Stat Med*. 2016;35:2561-2573.
36. Khanji C, Lalonde L, Bareil C, Lussier MT, Perreault S, Schnitzer ME. LASSO regression for the prediction of intermediate outcomes related to cardiovascular disease prevention using the TRANSIT quality indicators. *Med Care*. 2019;57:63-72.
37. Torres CL, Miller JW, Rogers QR. Determination of free and total cyst(e)ine in plasma of dogs and cats. *Vet Clin Pathol*. 2004;33:228-233.
38. Neumann S, Welling H, Thuere S. Evaluation of serum L-phenylalanine concentration as indicator of liver disease in dogs: a pilot study. *J Am Anim Hosp Assoc*. 2007;43:193-200.
39. Strombeck DR, Harrold D, Rogers Q, Wheeldon E, Stern J, Schaeffer M. Plasma amino acid, glucagon, and insulin concentrations in dogs with nitrosamine-induced hepatic disease. *Am J Vet Res*. 1983;44:2028-2036.

40. Strombeck DR, Rogers Q. Plasma amino acid concentrations in dogs with hepatic disease. *J Am Vet Med Assoc*. 1978;173:93-96.
41. Kathrani A, Allenspach K, Fascetti AJ, Larsen JA, Hall EJ. Alterations in serum amino acid concentrations in dogs with protein-losing enteropathy. *J Vet Intern Med*. 2018;32:1026-1032.
42. Tamura Y, Ohta H, Kagawa Y, et al. Plasma amino acid profiles in dogs with inflammatory bowel disease. *J Vet Intern Med*. 2019;33:1602-1607.
43. Benvenuti E, Pierini A, Gori E, et al. Serum amino acid profile in 51 dogs with immunosuppressant-responsive enteropathy (IRE): a pilot study on clinical aspects and outcomes. *BMC Vet Res*. 2020;16:117. doi:10.1186/s12917-020-02334-2
44. Chan DL, Rozanski EA, Freeman LM. Relationship among plasma amino acids, C-reactive protein, illness severity, and outcome in critically ill dogs. *J Vet Intern Med*. 2009;23:559-563.
45. Parker VJ, Fascetti AJ, Klamer BG. Amino acid status in dogs with protein-losing nephropathy. *J Vet Intern Med*. 2019;33:680-685.
46. Utsugi S, Azuma K, Osaki T, et al. Analysis of plasma free amino acid profiles in canine brain tumors. *Biomed Rep*. 2017;6:195-200.
47. Azuma K, Osaki T, Tsuka T, Imagawa T, Minami S, Okamoto Y. Plasma free amino acid profiles of canine mammary gland tumors. *J Vet Sci*. 2012;13:433-436.
48. Bevier DE, Miller MA, Rohleder JJ, Wozniak AD. Pathology in practice. Superficial necrolytic dermatitis. *J Am Vet Med Assoc*. 2010;237:365-367.
49. Brenseke BM, Belz KM, Saunders GK. Pathology in practice. Superficial necrolytic dermatitis and nodular hepatopathy (lesions consistent with hepatocutaneous syndrome). *J Am Vet Med Assoc*. 2011;238:445-447.
50. McEwen BJ. Superficial necrolytic dermatitis (hepatocutaneous syndrome) in a dog. *Can Vet J*. 1994;35:53-54.
51. Veterinary Clinical Pathology Laboratory. *Tips, Tricks, and Frequently Asked Questions*. UC Davis School of Veterinary Medicine; 2024. Accessed September 23, 2024. https://www.vetmed.ucdavis.edu/labs/aal-faqs

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Leela-arporn R, DeMarle KB, Heinze CR, Webster CRL. Plasma amino acid profiles of dogs with the hepatocutaneous syndrome and dogs with other chronic liver diseases. *J Vet Intern Med*. 2025;39(1):e17285. doi:10.1111/jvim.17285