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Alterations in serum amino acid concentrations in dogs with protein-losing enteropathy

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Background: Certain amino acids are decreased in humans with inflammatory bowel disease (IBD) and supplementation with the same amino acids has shown beneficial effects in animal models of IBD. Currently, the amino acid status of dogs with protein-losing enteropathy (PLE) is unknown.

Hypothesis/Objective: To determine if serum amino acid concentrations are abnormal in dogs with PLE and correlated with clinical and laboratory variables and outcome.

Animals: Thirty client-owned dogs diagnosed with PLE and 12 apparently healthy dogs seen at Bristol Veterinary School.

Methods: Retrospective study using stored residual serum from fasted dogs with PLE, collected at the time of diagnostic investigation and from apparently healthy dogs. Serum was analyzed for 30 amino acids using an automated high-performance liquid chromatography amino acid analyzer.

Results: Serum tryptophan concentrations were significantly decreased in dogs with PLE (median, 22 nmol/mL; range, 1–80 nmol/mL) compared with apparently healthy control dogs (median, 77.5 nmol/mL; range, 42–135 nmol/mL, $P < .001$). There were no significant differences in the remaining 29 serum amino acids between dogs with PLE and apparently healthy. Serum tryptophan concentrations were also significantly correlated with serum albumin concentrations in dogs with PLE ($P = .001$, $R^2 = 0.506$).

Conclusions and Clinical Importance: Decreased serum tryptophan concentration might play a role in the pathogenesis of canine PLE or be a consequence of the disease.

KEYWORDS
albumin, canine, intestine, tryptophan

1 | INTRODUCTION

There is a role for amino acids in gastrointestinal (GI) health. Amino acids such as arginine, glutamine, glycine, cysteine, *N*-acetylcysteine, and proline have functions in the GI tract such as attenuation of gut damage, support of intestinal barrier function and integrity, reduction in oxidative stress, restoration of mucosal immune homeostasis, and optimization of function by normalizing or reducing inflammatory cytokine secretion and increasing immune regulatory cytokine concentrations.[1–4] Recent studies have increasingly focused on the role of amino acids in the pathogenesis and treatment of humans with inflammatory bowel disease (IBD) and animal models of this disease.

There are altered serum amino acid concentrations in humans and animal models of IBD.[5,6] After 7 days of dextran sodium sulfate (DSS) induced colitis, mice had decreased concentrations of tryptophan, glutamic acid and aspartic acid.[6] In addition, tryptophan-deficient mice had more severe colitis when it was induced with DSS.[7] Humans with ulcerative colitis, a type of IBD, have decreased serum concentrations

of glutamic acid, glutamine, methionine, tryptophan and histidine compared with healthy controls.[8] Similarly, other studies have confirmed low serum tryptophan concentrations in humans with IBD.[5,9,10] There is an anti-inflammatory effect of dietary intervention with tryptophan or tryptophan metabolites in experimental models of colitis, with supplementation ameliorating clinical signs, improving weight gain and histological scores and decreasing gut permeability and expression of pro-inflammatory cytokines.[11,12] Similarly, supplementation with glutamine, arginine, N-acetylcysteine, and glycine in animal models of IBD has beneficial effects such as reduced intestinal permeability and decreased production of proinflammatory cytokines.[13–16]

Dogs with protein-losing enteropathy (PLE) have increased loss of protein from the intestinal tract resulting in hypoproteinemia. These animals likely also have a higher requirement for dietary protein because of increased demand from ongoing inflammation. Therefore, dogs with PLE might be at increased risk of developing an essential amino acid deficiency, especially as they might not be able to replenish their increased losses or meet their increased demands because of hyporexia or anorexia caused by their disease. Therefore, given the important functions of certain amino acids in the GI tract, a deficiency might contribute to or exacerbate disease in these dogs.

The aims of our study were to determine, (1) whether serum amino acid concentrations in dogs with PLE differ from healthy dogs and (2) to correlate any significant changes with age at diagnosis, clinical signs, and canine chronic enteropathy activity index (CCEAI)[17]; laboratory variables, and survival.

2 | MATERIALS AND METHODS

2.1 | Retrospective study criteria for case selection

The medical records at the Bristol Veterinary School were searched for dogs presented between January 2012 and October 2017 with a diagnosis of PLE. All medical records then were reviewed by one of the authors (A. Kathrani). Those dogs that had panhypoproteinemia with hypocholesterolemia but did not have histopathology of GI biopsies performed were still included. In addition, dogs that had solely hypoalbuminemia required appropriate diagnostic investigation including urine protein creatinine ratio to rule out other causes and histopathology of GI biopsies to confirm an underlying etiology consistent with PLE for inclusion into the study. The minimum diagnostic evaluation performed on each dog included a complete blood count, serum biochemistry, serum cobalamin, and folate concentrations and trans-abdominal ultrasound examination. Of the 30 dogs included in the PLE group, the following number had additional diagnostic procedures performed when indicated by the history, physical examination, and ultrasound examination findings: collection of intestinal biopsy specimens by upper GI endoscopy in 28 dogs (93%), with an additional 12 dogs (40%) having lower GI endoscopy, pancreatic testing (canine pancreatic lipase immunoreactivity in 15 dogs [50%] and trypsin-like immunoreactivity in 18 [60%]), basal cortisol concentration or ACTH stimulation test in 20 dogs (67%), preprandial or preprandial and postprandial bile acid concentrations in 20 dogs (67%), fecal parasitology using zinc sulfate flotation

with centrifugation in 24 dogs (80%), fecal culture (for Salmonella, Campylobacter, and Clostridium difficile) in 22 dogs (73%), empirical deworming in 27 dogs (90%) and urine protein creatinine ratio in 24 dogs (80%).

A fasted serum sample stored in −20°C from the time of diagnostic investigation for each dog was retrieved from the archive at Bristol Veterinary School and sent to the Amino Acid Laboratory at the University of California, Davis on dry ice for serum amino acid analysis.

2.2 | Data collection

Medical records were reviewed for each dog and the signalment; clinical history including duration of clinical signs, appetite, lethargy, weight loss, vomiting, and diarrhea; complete diet history; BCS; and, results of diagnostic tests including laboratory findings, transabdominal ultrasound examination findings, endoscopic findings, and histopathology report were collected. The CCEAI was calculated for dogs with chronic inflammatory enteropathy.[17]

To determine if the diet each dog was consuming complied with the recommendations of the World Small Animal Veterinary Association (WSAVA) Global Nutrition Committee, each manufacturer was contacted and asked to reply to the 8 questions outlined in section A of the web-link (WSAVA Global Nutrition Committee Guidelines: http://www.wsava.org/sites/default/files/Recommendations%20on%20Selecting%20Pet%20Foods.pdf). Diets were considered to meet the recommendations outlined by the WSAVA Global Nutrition Committee if the manufacturer could satisfactorily address all of the questions.

2.3 | Selection of control dogs

Serum samples from 12 dogs from which food was withheld for 10–12 hours, which were considered to be apparently healthy based on history, physical examination, complete blood count, and biochemistry panel were selected as controls. Dogs that were evaluated as apparently healthy were blood donors (4 dogs) or undergoing routine dental procedures (8 dogs). Serum was stored in −20°C for a similar time period as the PLE dogs and was retrieved from the archive at the Bristol Veterinary School and sent to the Amino Acid Laboratory at the University of California, Davis on dry ice for serum amino acid analysis.

2.4 | Serum amino acid analysis

Serum concentrations of taurine, L-aspartic acid, L-threonine, L-serine, L-asparagine, L-glutamic acid, L-glutamine, glycine, L-alanine, L-citrulline, L-α-amino-n-butyric acid, L-valine, L-cysteine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, β-alanine, L-phenylalanine, D-hydroxylysine, L-ornithine, L-lysine, 1-methyl-L-histidine, L-histidine, tryptophan, 3-methyl-L-histidine, L-carnosine, L-arginine, L-hydroxyproline, and L-proline were analyzed with an automated high-performance liquid chromatography amino acid analyzer (Biochrom 30, Biochrom Ltd, Holliston, Massachusetts), using a method described elsewhere.[18]

The R^2 of calculated standard curves of major amino acids were >0.9995. Internal standard recovery range of each sample was within 97%-100% and relative variances of major amino acids between duplicates were <2%. The detection limit of major amino acids was 0.1

nmol/mL with 50 μL load and recovery rates of spiking standards (addition of 200 nmol/mL) were 97%-102%.

2.5 | Ethical considerations

Stored residual blood was used in our study and the University of Bristol granted ethical approval for the study (VIN/17/026).

2.6 | Data analysis and statistics

Analyses were performed using a computer software package (IBM SPSS Statistics Version 23). The Mann-Whitney U test and Fisher's exact test were used to determine if there were any significant differences in age and sex/neuter status between the PLE and apparently healthy dogs, respectively.

The Shapiro-Wilk test was used to determine if the concentrations of each of the 30 amino acids were normally distributed. For those amino acids that were normally distributed, a t-test was used to determine if there were any significant differences between concentrations in the dogs with PLE versus the apparently healthy dogs. For amino acids that were not normally distributed, a Mann-Whitney U test was used to determine if there were any significant differences between concentrations in the dogs with PLE versus the apparently healthy dogs. A Bonferroni correction was applied, so that significance was defined as $P < .0017$.

For those amino acids that were significantly different between dogs with PLE and those that were apparently healthy, a simple logistic regression analysis was used to determine if there were any significant associations between the amino acid concentrations in the PLE dogs and age at diagnosis; chronicity of clinical signs; appetite, percentage body weight loss; body condition score (BCS); serum albumin, globulin, cholesterol, cobalamin, and folate concentrations; CCEAI; whether the diet consumed at diagnosis met the WSAVA Global Nutrition Committee Guidelines, survival versus death because of PLE, days to death or euthanasia because of PLE and serum sample storage time in −20℃. Variables associated with the amino acid concentrations in the PLE dogs with $P < .2$ in simple linear regression were entered into multivariable analyses. In the multivariable regression models, analyses were performed in a backward stepwise manner. All variables with $P < .2$ were initially included, and the variable with the highest P-value was removed until all remaining variables had a $P < .05$.

3 | RESULTS

3.1 | Dogs

Thirty dogs with PLE were included in the study: 1 intact male, 14 neutered males, and 15 neutered females. The age of the dogs ranged from 1.2 to 13.5 years, with a median age of 7.9 years. Breeds included cross breed (3), Yorkshire Terrier (2), Staffordshire Bull Terrier (2), Labrador Retriever (2), Lurcher (2), Shetland Sheepdog (2), Cocker Spaniel (2), Jack Russell Terrier (2), English Bulldog (2), and 1 each of the following breeds: Japanese Akita, Great Dane, Cavalier King Charles Spaniel, Border Terrier, Newfoundland, Sloughi, German

Shepherd Dog, Border Collie, Hungarian Vizsla, Greyhound, and a West Highland White Terrier. Body condition score ranged from 1/9 to 7–8/9, with a median of 3–4/9.

Twelve dogs that were apparently healthy were included in the study: 2 intact males, 4 neutered males, 1 intact female, and 5 neutered females. The age of the dogs ranged from 1 to 12 years, with a median of 8.5 years. Breeds included Cross Breed (3), Labrador Retriever (3), Greyhound (2), and 1 each of the following breeds: English Springer Spaniel, Border Terrier, Border Collie, and Siberian Husky.

There were no significant differences in age and sex/neuter status between the PLE and apparently healthy dogs ($P > .15$).

3.2 | History

Duration of clinical signs for all PLE dogs ranged between 1 day and 570 days (median, 60 days). Twenty dogs (67%) were reported to have lost weight (median 9.35% body weight loss, range 4%-28%), 2 dogs (7%) were reported to be weight stable, and weight changes were unreported for 8 dogs (27%). Four dogs (13%) were anorexic, 10 dogs (33%) were hyporexic, 11 dogs (37%) had an unchanged appetite, 3 (10%) were reported to be polyphagic, and appetite was unreported in 2 (7%) at the time of presentation. Twenty-seven (90%) dogs were reported to have diarrhea and 3 (10%) were reported to have normal feces at the time of presentation. Thirteen dogs (43%) were reported to be vomiting and for 17 (57%) vomiting was not a feature of their disease. Twenty dogs (67%) were eating a diet that met the recommendations outlined by the WSAVA Global Nutrition Committee at the time of presentation whereas 6 dogs (20%) were not and diet history was unreported in 4 dogs (13%). The median CCEAI was 8 with a range of 3–15.

3.3 | Histologic diagnosis

Twenty-eight of the 30 PLE dogs (93%) had histopathology of intestinal biopsies performed. All biopsy specimens were collected using endoscopy. One dog was diagnosed with small cell lymphoma and 1 dog with primary lymphangiectasia. Twenty-six dogs (87%) were diagnosed with chronic inflammatory enteropathy; 8 (27%) had lymphoplasmacytic and eosinophilic enteritis, 6 (20%) had lymphoplasmacytic enteritis, 3 (10%) had eosinophilic enteritis, 3 (10%) had lymphoplasmacytic, eosinophilic, and neutrophilic enteritis, 3 (10%) had plasmacytic and eosinophilic enteritis, 2 (7%) had lymphoplasmacytic and neutrophilic enteritis, and 1 (3%) had plasmacytic enteritis. Ten of the 26 dogs (38%) with chronic inflammatory enteropathy had concurrent lacteal dilatation on histopathology and 5 (19%) had crypt abscesses. Of the 2 dogs with PLE that did not have intestinal biopsies performed, one was empirically treated with prednisolone and chlorambucil as the owners declined endoscopy and the 2nd dog was diagnosed with salmonellosis on fecal culture.

Colonic biopsies were performed in 12 dogs (40%); 5 (17%) had lymphoplasmacytic colitis, 3 (10%) were within normal limits, and 1 had each of the following (3%); lymphocytic, lymphoplasmacytic, and eosinophilic, lymphoplasmacytic and neutrophilic, and plasmacytic and eosinophilic.

3.4 | Serum albumin, globulin, cholesterol, cobalamin, and folate concentrations

All dogs with PLE had hypoalbuminemia (median, 17.4 g/L; range, 11.7–29.3 g/L; reference range, 32–38 g/L), consistent with inclusion criteria. Twenty-one dogs (70%) had hypoglobulinemia, 8 dogs (27%) had globulin concentrations within the reference range, and 1 dog (3%) had concentrations above the reference range (median, 16.5 g/L; range, 9.6–36.8 g/L; reference range, 20–35 g/L). Nineteen dogs (63%) had hypocholesterolemia, 5 dogs (17%) had cholesterol within the reference range, and cholesterol was not measured in 6 dogs (20%; mean, 2.75 mmol/L; SD, 1.08 mmol/L; reference range, 3.5–7.0 mmol/L). Sixteen dogs (53%) had cobalamin concentrations below the reference range, 10 dogs (33%) had concentrations within the reference range, and 4 (13%) were above the reference range; 3 of the latter dogs had received parenteral cyanocobalamin before analysis (median, 193.5 pmol/L; range, 109–1476 g/L; reference range, 200–408 g/L). Fourteen dogs (47%) had folate concentrations below the reference range, 9 dogs (30%) had concentrations within the reference range, 6 (20%) were above the reference range, and 1 dog (3%) did not have a measurement performed (mean, 17.0 nmol/L; SD, 10.56 nmol/L; reference range, 12.0–30.0 nmol/L).

3.5 | Outcome

Thirteen of the 30 PLE dogs (43%) died or were euthanized because of the disease; 2 died (7%) and 11 (37%) were euthanized. Two (7%) were euthanized before discharge from the hospital and 11 (37%) were euthanized or died after (median days of survival after discharge from hospital, 18; range 6–180 days). Four of the 30 PLE dogs (13%) were euthanized because of diseases unrelated to their PLE; 2 of these dogs were euthanized 4 years and 1 dog 1.8 years after initial discharge from the hospital. Thirteen of the PLE dogs (43%) were still alive at the time of our study (median, 0.6 years; range 0.1–4.0 years).

3.6 | Serum amino acid concentrations

Serum tryptophan concentrations were significantly lower in dogs with PLE compared with apparently healthy dogs (PLE: median, 22 nmol/mL; range, 1–80 nmol/mL; apparently healthy: median, 77.5 nmol/mL; range, 42–135 nmol/mL, $P < .001$, Table 1, and Figure 1).

There were no significant differences in the remaining 29 serum amino acids between dogs with PLE and apparently healthy (Table 1).

3.7 | Correlation of serum tryptophan concentrations with various variables in the PLE dogs

In the simple linear regression analyses, age at diagnosis; appetite; percentage body weight loss; BCS; serum folate concentration; whether the diet consumed at diagnosis met the WSAVA Global Nutrition Committee Guidelines; survival versus death because of PLE; days to death or euthanasia because of PLE and serum sample storage time in −20°C did not significantly affect serum tryptophan concentrations in dogs with PLE ($P > .2$). The following variables were significantly correlated

with serum tryptophan concentrations in dogs with PLE in the simple linear regression models: chronicity of clinical signs ($P = .020$); serum albumin concentration ($P < .001$); serum globulin concentration ($P = .012$); serum cholesterol concentration ($P = .037$); serum cobalamin concentration ($P = .015$); and CCEAI ($P = .025$). Therefore, these variables, together with serum folate concentration as this variable had a P-value of $<.2$ in the univariable analysis were analyzed further in the multiple linear regression models. The multiple linear regression models showed that only serum albumin concentration was significantly correlated with serum tryptophan concentration in dogs with PLE ($P = .001$, R^2 [coefficient of determination from linear regression analysis] = 0.506) (Figure 2).

4 | DISCUSSION

In our study, serum tryptophan concentrations were significantly decreased in 30 dogs with PLE compared with 12 apparently healthy dogs. Tryptophan is a dietary essential amino acid in dogs; it is important for protein synthesis as well as serving as a precursor for additional bioactive compounds such as kynurenine, serotonin, melatonin, and picolinic acid.[19] The kynurenine pathway comprises at least 90% of tryptophan catabolism.[19,20] The enzyme indoleamine 2,3, dioxygenase 1 (IDO-1) is the initial rate-limiting step in the pathway for the oxidation of tryptophan to kynurenine.[19] Increased IDO-1 expression in human IBD is associated with lower serum tryptophan concentrations and a higher serum kynurenine tryptophan ratio because of increased tryptophan catabolism.[9] Therefore, decreased serum tryptophan concentrations in dogs with PLE in our study might be because of a similar mechanism of increased IDO-1 expression in the intestinal tract resulting in increased catabolism of tryptophan. However, further studies measuring concurrent serum kynurenine in dogs with PLE and expression of IDO-1 in intestinal biopsies would be needed to confirm this mechanism as the cause of decreased serum tryptophan concentrations in dogs with PLE.

Studies have also shown an association between serum tryptophan concentrations and disease activity in humans with IBD.[5,9] Although our study did not document an association between CCEAI or outcome and serum tryptophan concentrations in PLE dogs, there was a significant correlation with serum albumin concentrations. Therefore, as serum albumin concentration is an indicator of the severity of loss from the GI tract, our study showed that serum tryptophan concentrations are worse in those dogs with PLE with increased loss and therefore could be an indicator of severity of inflammation at the level of the intestinal tract because of increased IDO-1 expression. However, additional studies would be needed to confirm this hypothesis. Although tryptophan is reversibly bound to albumin in serum resulting in bound and free fractions, our study measured only free tryptophan in serum and therefore is unlikely to have been directly affected by the serum albumin concentrations. In addition, serum concentrations of free tryptophan in humans with chronic renal failure were shown to be uninfluenced by serum protein concentrations and the free fraction was normal in those patients with hypoalbuminemia.[21] Therefore, our study

TABLE 1 Serum amino acid concentrations in dogs with PLE and apparently healthy dogs

Serum amino acid	Apparently healthy median (range) or mean (SD)	PLE median (range) or mean (SD)	P-value
Tryptophan (nmol/mL)	77.5 (42–135)	22 (1–80)	<.001
Taurine (nmol/mL)	178 (110–272)	169 (70–404)	.48
L-Aspartic acid (nmol/mL)	27.5 (6–152)	21.5 (7–287)	.75
L-Threonine (nmol/mL)	296.5 (93.34)	299.4 (117.77)	.94
L-Serine (nmol/mL)	174 (87–403)	160.5 (83–584)	1.00
L-Asparagine (nmol/mL)	49.5 (14–575)	54.5 (20–172)	.36
L-Glutamic acid (nmol/mL)	102.5 (71–425)	97.5 (35–502)	.30
L-Glutamine (nmol/mL)	586.83 (303.67)	415.3 (265.98)	.08
Glycine (nmol/mL)	286 (176–534)	233 (128–537)	.13
L-Alanine (nmol/mL)	580 (363-1,235)	559.5 (195-1,424)	.67
L-Citrulline (nmol/mL)	51.5 (18–174)	26.5 (10–137)	.02
L-α-Amino-n-butyric acid (nmol/mL)	16 (5–56)	31 (1–122)	.04
L-Valine (nmol/mL)	222.5 (123–446)	275.5 (125–624)	.54
L-Cysteine (nmol/mL)	0.5 (0–5)	0 (0–8)	.24
L-Methionine (nmol/mL)	29 (1–71)	36.5 (0–129)	.37
L-Isoleucine (nmol/mL)	82.5 (44–163)	95 (50–250)	.21
L-Leucine (nmol/mL)	169 (100–828)	183 (79–1,015)	.77
L-Tyrosine (nmol/mL)	64.5 (39–254)	50.5 (12–338)	.21
β-Alanine (nmol/mL)	2 (0–38)	3 (0–33)	.83
L-Phenylalanine (nmol/mL)	74 (38–184)	80 (24–277)	.79
D-Hydroxylysine (nmol/mL)	2.5 (0–22)	3 (0–13)	.77
L-Ornithine (nmol/mL)	17 (12–82)	25.5 (6–133)	.33
L-Lysine (nmol/mL)	186 (77-2,262)	234 (21-1,873)	.82
1-Methyl-L-histidine (nmol/mL)	14 (5–157)	26 (6–163)	.25
L-Histidine (nmol/mL)	88.9 (17.28)	78.6 (31.70)	.30
3-Methyl-L-histidine (nmol/mL)	11.6 (5.21)	16.9 (7.78)	.04
L-Carnosine (nmol/mL)	33.5 (13–152)	25.5 (6–112)	.47
L-Arginine (nmol/mL)	210 (110-1,969)	191 (34-1,450)	.40
L-Hydroxyproline (nmol/mL)	36 (0–127)	11.5 (0–57)	.005
Proline (nmol/mL)	270 (146–767)	186 (67–744)	.07

Serum amino acid concentrations in 30 dogs with PLE and 12 apparently healthy dogs; median and range are listed for those amino acids that were not normally distributed and mean and SD is listed for those amino acids that were normally distributed for both groups. P-values obtained from t-test and Mann-Whitney U test for those amino acids that were normally distributed and not normally distributed, respectively between PLE dogs and apparently, healthy dogs are listed. A Bonferroni correction was applied, so that significance was defined as $P < .0017$.

demonstrated that in dogs with PLE, as serum albumin decreases, serum tryptophan concentrations decrease. Decreasing albumin concentrations are typically associated with more severe disease in dogs with PLE. Therefore, serum tryptophan concentration might also be an indicator of disease severity

An additional possibility for decreased serum tryptophan concentrations in dogs with PLE in our study could have been because of the diet the dogs were consuming. Unfortunately, the correlation between dietary tryptophan intake and serum concentrations was not able to be determined in our study, however, further studies assessing the serum kynurenine tryptophan ratio might help to avert any potential bias generated by differences in individual dietary intake.[22,23] Another important limitation of our study includes the absence of published reference ranges for serum amino acids in dogs. Serum was chosen to measure
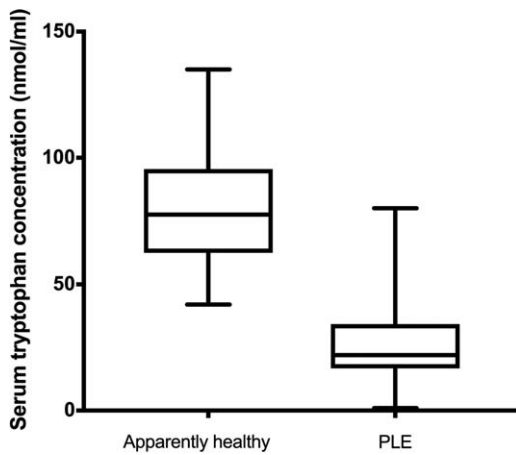
FIGURE 1　Box and whisker plot of serum tryptophan concentrations at the time of diagnostic investigation in dogs with PLE and apparently healthy. Apparently healthy: minimum = 42 nmol/mL, maximum = 135 nmol/mL, median = 77.5 nmol/mL, range = 93 nmol/mL, and interquartile range = 33 nmol/mL. PLE: minimum = 1 nmol/mL, maximum = 80 nmol/mL, median = 22 nmol/mL, range = 79 nmol/mL, and interquartile range = 17.50 nmol/mL. Serum tryptophan was significantly decreased in dogs with PLE compared with apparently healthy control dogs ($P < .001$)

amino acids in PLE dogs because of similar studies performed in human IBD, which had documented significant changes in amino acids. Therefore, a control group consisting of 12 apparently healthy dogs was used for comparison. Unfortunately, because of the small number of control dogs used, we were unable to breed-match them to the PLE group. Although a recent study documented no statistically significant changes in blood tryptophan concentrations between small and larger dogs,[24] further studies should focus on a breed-matched control group to remove any possible confounding effects from breed variation. Although the role of prolonged freezing on serum amino acids in dogs is also unknown, the dogs in the apparently healthy group had serum stored in −20°C over a similar time period as dogs in the PLE group and none of the remaining 29 amino acids were shown to be significantly different between the 2 groups. One study documented that storage of human serum at −20°C for up to 26 weeks did not significantly affect serum tryptophan concentrations.[25] A study reported a mean and standard deviation (SD) of serum tryptophan concentrations using high performance liquid chromatography from samples that had been frozen for a shorter duration (5 months) in 9 clinically healthy bitches of 5 different breeds as 68.44 and 21.77 nmol/mL, which was not dissimilar to the results obtained in our apparently healthy group, using frozen serum stored for a longer duration.[26] However, further studies are needed to determine the effect of prolonged storage in −20°C. Another limitation of our study involved the retrospective study design, which could have made the interpretation of the clinical history and assignment of the CCEAI score inconsistent because of multiple clinician involvement in the cases. In addition, some variables such as serum cholesterol concentrations or appetite were not assessed or reported in all cases and 2 dogs did not have intestinal biopsies performed to definitively diagnose the underlying cause for

the PLE. Therefore, a prospective study to measure serum amino acids in dogs with PLE would allow consistent assessment of the clinical history for all cases and complete historical and diagnostic information as well as negating any potential effect of prolonged storage of serum on amino acid concentrations. Furthermore, although the majority of PLE cases in our study had a chronic inflammatory enteropathy, additional etiologies were also included; therefore, future studies should focus on serum tryptophan concentrations in PLE dogs with similar etiologies to determine if this is influenced by the underlying pathology.

Although our study did not document a significant change in concentrations of other serum amino acid between dogs with PLE and apparently healthy, this might have been because a conservative test to adjust for multiple comparisons was used resulting in a stringent P-value to minimize Type 2 error rates. Therefore, future studies should also focus on those amino acids, such as L-hydroxproline that might have reached significance if a less conservative test was used, especially in dogs with similar etiologies of PLE and breed-matched control dogs to determine if they play a role in disease pathogenesis.

In conclusion, we documented significantly decreased tryptophan concentrations in dogs with PLE compared with apparently healthy dogs. In addition, serum tryptophan concentrations were significantly correlated with serum albumin concentrations in dogs with PLE. Additional studies should be performed to determine serum kynurenine tryptophan ratio in dogs with PLE to negate any effects from dietary intake and IDO-1 expression in the intestinal tract to determine the underlying pathogenesis. In addition, further studies are needed to determine if correction of serum tryptophan concentrations might improve clinical, laboratory or outcome variables in dogs with PLE.
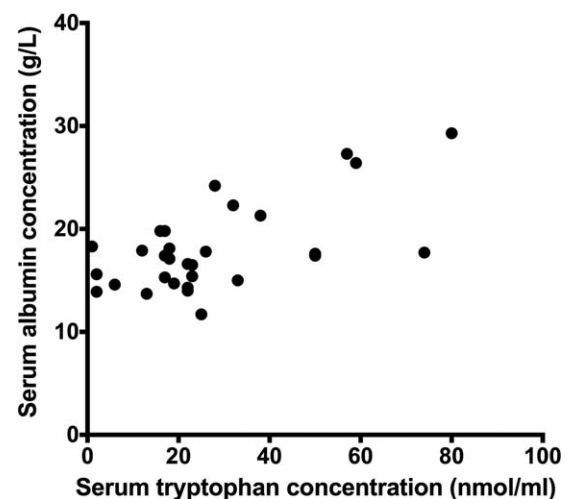

FIGURE 2　Scatter dot plot of serum tryptophan concentrations (nmol/mL) and serum albumin concentrations (g/L) at the time of diagnostic investigation in 30 dogs with PLE. Serum tryptophan concentrations were significantly correlated with serum albumin concentrations in dogs with PLE ($P = .001$, R^2 [coefficient of determination from linear regression analysis] = 0.506)

CONFLICT OF INTEREST DECLARATION

A.J. Fascetti is the Scientific Director of the Amino Acid Laboratory at the University of California Davis that provides amino acid analysis on a fee for service basis. This did not lead to any conflict of interest or influenced the collection or interpretation of results.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

The University of Bristol granted ethical approval for the study (VIN/17/026).

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