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Amino acid profiles for red wolves (*Canis rufus*) managed under human care are significantly different compared to the profiles of domestic dogs (*Canis familiaris*)

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OBJECTIVE
To establish a reference interval for amino acid profiles for healthy red wolves (*Canis rufus*).

METHODS
Heparinized plasma of 48 red wolves was collected between August 2023 and April 2024 and sent to the University of California-Davis Amino Acid Laboratory for analysis. Reference intervals were created using the published American Society for Veterinary Clinical Pathology reference interval guidelines. Data were analyzed via Gaussian data distribution, and parametric statistical methods were used to produce a 90% CI of reference limits. The means of the red wolf intervals created were compared to those of the domestic dog using a *z* test.

RESULTS
Reference intervals were created for red wolves (n = 48). Upon completion of the *z* test, 11 of 21 amino acids were found to be statistically significantly different compared to those of the domestic dog.

CONCLUSIONS
A reference interval was created for red wolves. The red wolf amino acid profiles are different than those of the domestic dog, with 52% (11/21) of the profiles being statistically different.

CLINICAL RELEVANCE
Red wolf amino acid profiles should not be compared to those of the domestic dog reference intervals due to the significant difference between profiles.

Keywords: red wolf, *Canis rufus*, amino acid, irritable bowel disease, captive

Amino acid profiles have been established for multiple domestic species, such as dogs and cats, over the years.[1–3] Specific amino acids, such as taurine, which is found to be important in growth and cardiovascular health, have been researched more readily than other amino acids.[1–5] However, many exotic species under human care have unknown complete amino acid profiles.[3–5] These amino acid profiles are helpful in our animals to assess overall health, as amino acids are essential building blocks of proteins within the body responsible for internal organ health/function, growth, and immunity.[6–8] Having a reference interval for amino acid profiles is imperative for helping red wolves under human care, as it can help determine their underlying need for further veterinary care and even help with conservation decisions to release. Amino acids are essential building blocks of protein and are important to the health of most body systems including the gastrointestinal, endocrine, immune, and musculoskeletal systems.[1,2] Changes in these panels could indicate possible disease states or support a diet change.[6,7]

Red wolves (*Canis rufus*) inhabit eastern North Carolina and are currently considered an International Union of Conservation of Nature critically endangered species with an estimate of only 20 to 30 wild mature individuals during the International Union of

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Conservation of Nature assessment in 2018.[9] There were approximately 267 wolves under human care as of October 2023 in the Saving Animals From Extinction (SAFE) program, which accounts for red wolves under human care across the world.[10] This species is known for having gastrointestinal issues, such as irritable bowel disease.[11,12]

The need for amino acid profile intervals was noted during an examination of a red wolf at North Carolina State College of Veterinary Medicine. An amino acid profile was run to further investigate prolonged anesthetic recoveries in this wolf after annual examinations in November of 2020.[13] The amino acid profile results had multiple concerning values compared to the published domestic dog reference interval; however, it was determined that the values could not be interpreted correctly without species-specific reference intervals (T. Harrison, DACZM, College of Veterinary Medicine, North Carolina State University, email, August 2021).[3] Furthermore, there was uncertainty as to what degree the diet of the red wolf impacted amino acid values. For example, in maned wolves, *Chrysocyon brachyurus*, cystinuria has been extensively researched and found to be linked to diet, helping institutions make diet recommendations based on this information to help better manage the condition of cystine stone formation.[14]

The experience with the wolf at the North Carolina facility spurred the question of what an amino acid profile would look like in healthy captive red wolves. There is research that has been previously published on red wolf irritable bowel disease, and many hypotheses exist on how to help better manage it, particularly with differing diet strategies and relationship to the gut microbiome.[11,12,15] Little to no research has been evaluated as far as amino acid supplementation in red wolves with gastrointestinal disease and if that would be a feasible endeavor to support gastrointestinal health.

The purpose of this study was to establish a reference interval for amino acid profiles for red wolves and compare those values to the amino acid reference intervals reported in domestic dogs.[3] The authors suspected that the amino acid profiles would differ from the domestic dog profile.

Methods

This study encompassed 3 different populations of red wolves housed at facilities in North Carolina (Museum of Life and Science, n = 2; North Carolina College of Veterinary Medicine, 6; and North Carolina Zoo, 17) as well as a fourth population in the state of Washington (Point Defiance Zoo and Aquarium, 23); all wolves were managed under the SAFE program through the Association of Zoos and Aquariums and the US Fish and Wildlife Service. All biological samples were collected and submitted for processing under a Designated Agent Letter through the US Fish and Wildlife Service, as well as under North Carolina State University IACUC No. 23-420.

Samples for this study were collected opportunistically where wolves had blood collected either during their annual examination or during an examination for preshipment or release from August 2023 to April 2024. All wolves had been fasted for 4 to 8 hours before sedation for their exam. Samples of 2 mL of heparinized blood were collected within

Table 1—Reference interval (RI) of amino acid (nmol/mL) for 48 red wolves (*Canis rufus*) including minimum, maximum, mean, SD, as well as upper and lower 90% CI limits including the domestic dog (*Canis familiaris*) RI.[3]

Amino acid	Mean	Median	Minimum	Maximum	SD	Upper 90% CI	Lower 90% CI	Domestic dog RI
Alanine	408.39	395.03	187.67	1,070.01	150.95	444.19	372.60	380–398
Arginine	77.99	80.71	16	166.26	28.38	84.72	71.26	99–105
Asparagine	58.97	55.46	24	151.76	22.37	64.33	53.61	40–42
Aspartic acid	9.36	7	3.40	58	9.36	11.24	8.04	6.8–7.2
Carnitine	33.85	30.67	13	78	12.99	36.93	30.77	24.4 ± 8.4
Citrulline	16.29	15	2.80	48	7.42	18.42	14.52	39–43
Glutamic acid	533.07	527.70	184	973.28	141.93	566.72	499.42	486–504
Glutamine	60.69	43.81	24.48	352	54.05	73.50	47.87	23–25
Glycine	226.48	218.03	100	557.75	68.44	242.70	210.25	258–274
Histidine	80.48	80.51	12	121.42	19.46	85.09	75.87	69–73
Hydroxyproline	34.25	30.73	2	160	29.04	41.28	27.21	63–71
Isoleucine	45.18	42.06	9	87.84	16.95	49.20	41.16	50–52
Leucine	92.65	86.21	41	175.08	33.62	100.62	84.68	117–123
Lysine	109.92	108	19	268.73	41.95	119.87	99.97	126–136
Methionine	45.31	44.58	10	93	15.10	48.97	41.66	55–59
Ornithine	13.88	11	5	51.52	9.92	16.26	11.50	33–37
Phenylalanine	55.90	56.68	5	122.14	15.70	59.62	52.18	44–46
Proline	121.77	111.36	64	287	52.89	134.31	109.23	241–257
Serine	128.55	120.5	69	277.04	33.38	136.47	120.64	104–110
Taurine	156.31	134.97	65	592	84.31	176.30	136.32	75–79
Threonine	155.82	152.26	87.99	261.43	38.68	166.81	144.82	173–183
Tryptophan	69.29	67.75	21	130	21.13	74.35	64.22	58–62
Tyrosine	38.75	37.82	6	75.15	12.79	41.78	35.72	38–40
Valine	138.62	128	90	247.19	32.78	146.39	130.85	154–162
L-methylhistidine	7.93	6.14	1	81	11.17	10.64	5.22	
3-Methyhistidine	7	10	3.72	16.24	3.41	10.57	8.33	5–7

a heparinized green top tube (BD Vacutainer Heparin Tubes; Thomas Scientific) and were centrifuged (2,000 X *g* or 450 to 530 rpm for 10 minutes; Eppendorf Labs;) to obtain at least 0.5 mL of heparinized plasma. The plasma was pipetted off and stored in a nonadditive tube (Flex Tube; Eppendorf Labs) within an ultralow freezer (−80 °C) until it was sent out as a batch submission overnight on ice to the University of California-Davis Amino Acid Laboratory for complete analysis. Once obtained, the laboratory added 6% sulfosalicylic acid (1:1) to the sample for deproteinization, and then the samples were centrifuged again (14,000 rpm for 25 minutes). The sample was then filtered through a 0.45-mL syringe drive polytetrafluoroethylene filter. The pH was adjusted to 2.2 for analysis, and then the sample was loaded into the Biochrom Bio 30+ amino acid analyzer (HB Harvard Bioscience).

Inclusion criteria were wolves in an overall healthy condition; therefore, their physical examination, medical records, CBC count, and chemistry profiles were evaluated before inclusion. Any wolf with clinical signs of gastrointestinal disease or liver disease or that appeared to have and maintain a low body condition score (< 2/5) was excluded from this study. Reference intervals were created using the published American Society for Veterinary Clinical Pathology reference interval guidelines.[16] Due to the small sample size (< 120 individuals), data were analyzed using Gaussian data distribution with parametric statistical methods to produce a 90% CI of reference limits.[16,17] The CI did not exceed 0.2 times the width of the reference interval.[16,17]

The compiled amino acid profiles were assessed for normality in JMP software, version 17 (SAS Institute), using a Shapiro-Wilk test before creating a reference interval for red wolves establishing minimum, maximum, mean, SD, as well as upper and lower 90% CI limits.

Upon completion of the reference intervals, the means of the red wolf population were compared to those of the domestic dog using the *z* test to evaluate if there was statistical significance between the 2 groups. This analysis was completed using the same JMP software. Only 21 of the 26 amino acids were able to be compared between red wolves and domestic dogs due to the availability of the mean values for each amino acid.[3]

Results

Amino acid results for red wolves are reported **(Table 1)**, including published domestic dog intervals for comparison.[3]

A total of 48 samples (19 females and 29 males) were included in this study. Forty-one of the wolves were fed a combined diet of dry kibble and whole prey items and/or Nebraska Classic Carnivore Diet (Central Nebraska Packing Inc), and 7 were fed a diet of whole prey only. Whole prey items included rodents (rats, mice), deer, rabbits, chicks, and quail. The crude protein percentage of most diet items is provided **(Table 2)**. Wolves on a hybrid diet were fed approximately 30% to 50% whole prey and/or Nebraska meat and 50% to 70% dry kibble.

All wolves were generally healthy at the time of blood collection. Of the wolves included in this study, 3 males were diagnosed with unilateral cryptorchid, 1 wolf had recovered from *Bartonella* 2 years prior, and 2 were undergoing treatment for dental disease (root canal and extractions due to previously fractured teeth). The wolves included in this study ranged from 1 to 13 years old, with a mean age of 4 years old.

A Shapiro-Wilks test was used to test for normality for all values, and a *P* value > 0.05 for all values.

Upon completion of the *z* test, there were 11 amino acids out of the 21 able to be compared that were found to be statistically different compared to the domestic dog.[3] Red wolves were found to be

Table 2—Crude protein represented by percentage of differing aspects of the diets of the red wolves (*C rufus*) used to create the amino acid RI.

Diet item	Crude protein (%)
Nebraska Classic Carnivore Diet[18]	19
Infinia Turkey and Sweet Potato Grain Free[19]	32
Hills Science Diet High Energy[20]	29.7
Hills Science Diet Adult Chicken and Barely[20]	21
Whole medium rat[21]	20.95
Whole medium mouse[21]	18.25
Whole large rabbit[21]	17.84
Whole medium chick[21]	16.6
Whole large quail[21]	24.74
Deer meat (whitetail deer)[22]	23.6

Most (85%) wolves in this study had a combination (approx 50%/50%) of whole prey items and/or Nebraska diet and dry kibble. Only 7 wolves (15%) were fed either exclusively whole prey or a combination of Nebraska diet and whole prey.

Table 3—Statistical significance by *z* test comparing the mean value of red wolves (*C rufus*) amino acid RIs to the mean value of domestic dogs.[3]

Amino acid	*z* Test	*P* value
Alanine	0.71	.238
Arginine	1.78	.037
Asparagine	1.91	.028
Citrulline	3.3	< .001
Glutamic acid	21.63	< .00001
Glutamine	18.4	< .00001
Glycine	1.84	.032
Histidine	0.8	.211
Hydroxyproline	3.2	< .001
Isoleucine	0.61	.27
Leucine	1.85	.032
Lysine	1.4	.081
Methionine	1.18	.119
Ornithine	3	< .01
Phenylalanine	1.1	.136
Proline	6.46	< .001
Serine	1.4	.081
Taurine	5.17	< .00001
Threonine	1.2	.115
Tryptophan	0.8	.211
Tyrosine	0	.5
Valine	1.05	.146

statistically higher for taurine ($P < .00001$), asparagine ($P < .028$), and glutamic acid ($P < .00001$) compared to domestic dogs **(Table 3)**. Red wolves were found to be lower for glutamine ($P < .00001$), glycine ($P < .032$), citrulline ($P < .001$), leucine ($P < .032$), ornithine ($P < .001$), arginine ($P < .037$), hydroxyproline ($P < .001$), and proline ($P < .00001$) compared to domestic dogs.

Discussion

This study created a reference interval for amino acid profiles in red wolves and compared the means of those values to those of the domestic dog interval.[3] A total of 11 red wolf amino acid values were found to statistically differ in comparison to the domestic dog values.[3] There could be many reasons why red wolves had statistically significant differences in amino acid values, which could include their diet (as all red wolves in this study had a portion of whole prey included in their diet), genetic differences compared to the domestic dog, or potential underlying and undiagnosed gastrointestinal disease.[11,12]

Cysteine and taurine have been the most common amino acids studied in wolves.[14,23–25] A previous study[23,24] reported that it was common to have lower cysteine levels, an amino acid that helps with cellular signaling and an antioxidant, in wolves than in domestic dogs. Wu[24] discussed that wolves most likely ingest sufficient amounts of cysteine; however, the domestic dog possesses a more efficient way to synthesize this amino acid, making their plasma concentrations higher.[23] In the current study, the authors were unfortunately unable to evaluate this specific amino acid in red wolves due to samples needing to be deproteinized within an hour postcollection. These samples collected were not and therefore values for the amino acid cysteine are not reported.

Four amino acids that had the highest significant difference between red wolves and dogs were glutamine, glutamic acid, taurine, and proline. Glutamine and glutamic acid have important roles in the urea cycle, while proline is used for collagen structure and function, neurologic development, and immunity.[24] Taurine is important in many bodily functions including digestion and the cardiovascular, musculoskeletal, and nervous systems.[24] Previous studies[26–28] in humans and other animals have found that concentrations of amino acids can differ intraindividually among age, sex, and body mass index. The authors of the current study speculate that many different factors can influence amino acid concentrations including time of year, animals getting ready for the winter months, periods of limited diet, and perhaps pregnancy or prepregnancy demands.[29] The most significant concern of red wolves under human care is gastrointestinal disease, specifically irritable bowel disease.[11,12,15] In humans, research has concluded that the most significant amino acids associated with gastrointestinal health include glutamine, glycine, and proline.[8,24,30] Glycine and proline could be monitored in red wolves in comparison to the red wolf intervals created in this study, and supplementation of glycine and proline could potentially improve red wolf gastrointestinal health. Most of the wolves in this study (85%) were on a combined whole prey and commercial domestic dog diet. Most wolves are considered facultative carnivores, and there is a potential concern that commercial dog diets have lower nutrients, such as crude protein, calcium, zinc, iron, and magnesium compared to wild-type diets.[29] There is further research that evaluated the amino acid profiles of domestic dogs with irritable bowel disease and/or food enteropathy and found that these dogs had significantly lower serine compared to control dogs.[31–33] Serine plays a large role in the construction of other amino acids as well as in gut health in humans as well as in canids.[24] The red wolf serine values found in this study were not statistically different when compared to domestic dogs, although analysis of wolves with severe disease could show differences.

Evaluating serine as a biomarker for irritable bowel disease is currently being investigated in humans, where serine has also been shown to be important in gut inflammation with multiple diseases.[24,34] Further research has been conducted to evaluate if serine proteases are valuable in supplementation for gut health for those afflicted with inflammatory diseases.[34] In weaning piglets, a study[35] evaluated supplementation of serine and found that there was a potential benefit to prevent gut dysregulation and inflammation caused by weaning. Additional research is needed to evaluate the potential benefits of serine supplementation in red wolves with irritable bowel disease.

Food enteropathy is another disorder that commonly causes gastrointestinal inflammation.[31,32] A study[32] evaluating dogs with food enteropathy found that dogs had lower asparagine, histidine, glycine, cystine, and leucine and higher phenylalanine. Red wolves in this study had lower glycine and leucine in comparison to domestic dogs. Lower glycine and leucine levels in wolves could be another area of important research for red wolves to determine if these values could serve as biomarkers for potential gastrointestinal disease.

The wolf that was evaluated originally by a North Carolina institution was a female red wolf that had prolonged anesthesia recovery events. In a comparison of the wolf's amino acid levels to those of the reference interval for red wolves created in this study, this individual had predominately elevated amino acid plasma concentrations, which could indicate inflammation or other disease.[6,7,31] There have been studies[36,37] in dogs that map liver disease and amino acid concentrations, such as dogs with hepatocellular carcinoma had elevated glutamic acid and dogs with chronic hepatitis had increased proline, phenylalanine, and tyrosine. This female wolf had a glutamic acid of 1,073 nmol/mL, which was almost twice the reference interval created for red wolves (reference interval, 499.42 to 566.72 nmol/mL). In addition, the wolf's proline was moderately elevated at 188 nmol/mL (reference interval, 109.23 to 134.31 nmol/mL), phenylalanine was markedly elevated at 715 nmol/

mL (reference interval, 52.18 to 59.62 nmol/mL), and tyrosine was markedly elevated at 516 nmol/mL (reference interval, 35.72 to 41.78 nmol/mL). A full comparison is provided **(Table 4)**. The authors suspect that this wolf did indeed have liver dysfunction; however, unfortunately, this was not confirmed due to the release of this wolf to the wild and loss of ability for follow-up; however, this case reflects the benefit of amino acid profile measurement among animals (T. Harrison, DACZM, College of Veterinary Medicine, North Carolina State University, email, August 2021).

In addition, both the dog amino acid reference interval and the red wolf amino acid reference interval were compiled by the same laboratory (University of California-Davis Amino Acid Laboratory); however, different analyzers were used.[3] The machine used to analyze the dog amino acid levels was the Beckman 6300; whereas, the machine used in this current study was the Biochrom Bio 30+.[3,38] The Biochrom Bio 30+ is the gold standard for amino acid analysis as it continues to perform the same methods as the Beckman 6300; however, it can evaluate more amino acids (56 total) and their derivatives.[38]

This is the first paper to evaluate the amino acid profiles in red wolves. However, there are multiple limitations in this study, including the inability to accurately obtain cysteine values. Additionally, although this study encompasses approximately one-fourth of the population under the SAFE program, it did not meet the minimum data requirements set forth by the American Society for Veterinary Clinical Pathology reference interval standards (n = 120), since this is a critically endangered species with limited numbers existing.[9,16,17] Another limitation of this study was not being able to compare animals under human care to wild red wolf populations. Additional evaluation of individuals released to the wild or born in the wild could be helpful to compare to healthy amino acid profiles. In addition, full profiles, not just cysteine or taurine, of other nondomesticated canid species under human care would be helpful to compare to red wolf values.[14,25,39,40]

In conclusion, this study found that the amino acid reference intervals for red wolves are significantly different than those of domestic dogs. Additional research is needed to determine the association between these amino acid profiles and gastrointestinal inflammatory diseases and if supplementation with one or more amino acids could potentially be helpful in those disease states.

Table 4—Comparison of amino acid profile of the female red wolf from North Carolina State University (T. Harrison, DACZM, College of Veterinary Medicine, North Carolina State University, email, August 2021) that had difficulties recovering from anesthetic events to the red wolf (*C rufus*) RIs created as well as published domestic dog (*C familiaris*) amino acid intervals.[3]

Amino acid	Female red wolf	Red wolf RI (nmol/mL)	Domestic dog RI (nmol/mL)
Alanine	505	373–444	380–398
Arginine	27	71–85	99–105
Asparagine	91	54–64	40–42
Aspartic acid	19	8–11	6.8–7.2
Carnitine		30–37	24.4 ± 8.4
Citrulline	84	14–18	39–43
Glutamic acid	1,073	499–567	486–504
Glutamine	161	48–74	23–25
Glycine	437	210–243	258–274
Histidine	253	75–85	69–73
Hydroxyproline	29	27–41	63–71
Isoleucine	40	41–49	50–52
Leucine	217	84–101	117–123
Lysine	79	99–120	126–136
Methionine	159	41–49	55–59
Ornithine	229	11–16	33–37
Phenylalanine	715	52–59	44–46
Proline	188	109–134	241–257
Serine	387	121–137	104–110
Taurine	31	136–176	75–79
Threonine	91	145–167	173–183
Tryptophan	97	64–74	58–62
Tyrosine	516	35–42	38–40
Valine	343	130–146	154–162
1-Methyhistidine	2	5–11	
3-Methylhistidine	2	8–11	5–7

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