

## SUPPLEMENTARY MATERIAL

**SUPPLEMENTARY TABLE 1.** Complete blood count and blood chemistry analyses in dogs consuming meat-based diets (baseline) versus plant-based nutrition (6 and 12 months). Values reflect median (minimum - maximum).

CBC values remained within normal reference intervals. In one dog, the total leukocyte count was within the normal interval, but the neutrophil count (2.5 K/ $\mu$ L) was below the lower end of the reference interval at 6 months, which normalized at 12 months. Standard blood chemistry values all remained within clinically unremarkable reference intervals. One dog had a borderline low phosphorous level (2.2 mg/dL) at the endpoint (12 months) in combination with a normal vitamin D level (237 nmol/L). The sodium level (130 mmol/L) measured below the minimum value of the reference interval at baseline in one dog, which normalized at 6 and 12 months. The chloride level was borderline low (108 mmol/L) in one dog at the endpoint. The albumin level (4.3 g/dL) measured above the maximum value of the reference interval in one dog at endpoint, while total protein remained within the normal reference interval. GGT (gamma-glutamyl transferase) baseline levels were above the maximum value of the reference interval in two dogs (13 and 19 U/L), which had normalized at 6 and 12 months. The bilirubin level (1.2 mg/dL) was mildly elevated at baseline in one dog, which normalized at 6 and 12 months. Osmolality measured below (268 mmol/kg) and above (313 mmol/kg) the reference interval in two dogs at baseline, but normalized at 6 and 12 months.

Parameter	Unit	Baseline	6 months	12 months	P-value (Friedman)	P-value (Wilcoxon)	Reference interval (IDEXX)
<i>CBC</i>							
Erythrocytes	M/ $\mu$ L	7.6 (6.8-8.5)	7.9 (6.8-8.7)	8.0 (7.4-8.9)	0.03	0.003	5.65-8.87
Hematocrit	%	52.0 (46.0-58.1)	53.1 (47.5-58.4)	54.1 (48.1-60.7)	0.002	0.007	37.3-61.7
Hemoglobin	g/dL	19.1 (16.6-20.5)	19.2 (17.3-20.7)	19.6 (17.9-21.2)	0.003	0.002	13.1-20.5
Leukocytes	K/ $\mu$ L	7.3 (5.2-11.9)	7.6 (4.8-13.3)	7.0 (5.0-12.1)	0.59	0.73	5.05-16.76
Neutrophils	K/ $\mu$ L	4.7 (3.6-9.1)	5.2 (2.5-10.5)	4.6 (3.4-8.8)	0.89	0.56	2.95-11.64
Lymphocytes	K/ $\mu$ L	1.7 (0.9-2.2)	1.8 (1.2-3.1)	1.7 (1.0-2.1)	0.30	0.22	1.05-5.10
Monocytes	K/ $\mu$ L	0.4 (0.2-0.9)	0.4 (0.2-0.8)	0.4 (0.3-0.9)	0.69	0.24	0.16-1.12
Eosinophils	K/ $\mu$ L	0.4 (0.3-1.0)	0.4 (0.2-1.0)	0.5 (0.3-1.2)	0.62	0.35	0.06-1.23
Basophils	K/ $\mu$ L	0.01 (0.00-0.09)	0.01 (0.00-0.08)	0.02 (0.00-0.06)	0.69	> 0.99	0.00-0.10
Platelets	K/ $\mu$ L	202 (144-322)	232 (140-400)	214 (185-318)	0.34	0.21	148-484
<i>Chemistry</i>							
Glucose	mg/dL	99 (76-109)	99 (75-109)	107 (85-117)	0.01	< 0.001	74-143
Creatinine	mg/dL	1.0 (0.6-1.4)	1.1 (0.8-1.4)	1.1 (0.7-1.3)	0.14	0.13	0.5-1.8
BUN	mg/dL	20 (9-23)	15 (6-23)	19 (6-27)	0.09	0.77	7-27

Phosphorus	mg/dL	4.4 (3.2-5.8)	4.0 (2.5-6.0)	3.9 (2.2-4.8)	0.08	0.01	2.5-6.8
Calcium	mg/dL	10.2 (9.8-10.6)	10.1 (9.6-10.6)	10.2 (9.1-10.5)	0.21	0.36	7.9-12.0
Sodium	mmol/L	153 (130-158)	153 (146-156)	150 (145-152)	0.002	0.01	144-160
Potassium	mmol/L	4.2 (3.6-5.0)	4.4 (3.8-5.5)	4.6 (4.2-5.5)	< 0.001	0.001	3.5-5.8
Chloride	mmol/L	117 (112-125)	115 (110-119)	113 (108-116)	0.03	0.004	109-122
Total Protein	g/dL	6.7 (6.0-7.4)	6.9 (6.1-7.7)	6.9 (6.1-7.4)	0.27	0.97	5.2-8.2
Albumin	g/dL	3.1 (2.7-3.5)	3.4 (3.0-3.9)	3.6 (3.1-4.3)	0.002	0.003	2.3-4.0
ALT	U/L	97 (30-129)	61 (22-115)	51 (21-357)	0.06	0.12	10-125
ALP	U/L	61 (13-222)	44 (22-222)	40 (10-192)	0.15	0.05	23-212
GGT	U/L	3 (0-19)	1 (0-7)	3 (0-6)	0.003	0.62	0-11
Bilirubin	mg/dL	0.4 (0.1-1.2)	0.4 (0.2-0.6)	0.2 (0.1-0.5)	0.02	0.006	0.0-0.9
Cholesterol	mg/dL	178 (121-290)	210 (111-288)	193 (131-280)	0.52	0.40	110-320
Osmolality	mmol/kg	303 (268-313)	303 (289-310)	301 (291-305)	0.24	0.12	290-310
T4	µg/dL	1.7 (0.9-2.6)	1.5 (1.0-3.7)	1.8 (1.1-3.7)	0.42	0.32	1.0-4.0

**SUPPLEMENTARY TABLE 2.** Urinalysis in dogs consuming meat-based diets (baseline) versus plant-based nutrition (6 and 12 months). Values refer to median (minimum - maximum). N/A: not applicable (since each row has zero difference, which precludes calculation of a paired test). Semi-quantitative UA parameters were tabulated as ‘0’ (normal/negative/not detected or <1/HPF), ‘1’ (trace), or the highest reported value (e.g., 1-5/HPF and 6-20/HPF were tabulated as ‘5’ and ‘20’, respectively), where HPF is high power field. Urine samples were collected via cystocentesis where iatrogenic microscopic hematuria is to be expected.

Urine pH trended downwards and towards normal - although findings did not reach statistical significance ( $p = 0.05$ ) - as levels were elevated in eight dogs at baseline (pH 8-9), four dogs at 6 months (pH 8) and two dogs at endpoint (pH 8). Potential crystal formation is associated with changes in urine pH. In this study, we identified a variety of crystals in the urine from a total of 60% of the dogs (9 of 15) at different time points (including 3 dogs at baseline, 3 different dogs at 6 months, and 3 different dogs at 12 months) with no identifiable pattern to the changes.

<b>Parameter</b>	<b>Baseline</b>	<b>6 months</b>	<b>12 months</b>	<b>P-value (Friedman)</b>	<b>P-value (Wilcoxon)</b>	<b>Normal Values</b>
Specific gravity	1.044 (1.020-1.050)	1.036 (1.015-1.050)	1.036 (1.015-1.050)	0.06	0.06	Variable
Glucose	0 (0-0)	0 (0-100)	0 (0-0)	0.37	N/A	Negative
Bilirubin	0 (0-1)	0 (0-1)	0 (0-1)	0.37	0.63	Negative
Ketones	0 (0-15)	0 (0-15)	0 (0-15)	0.20	N/A	Negative
pH	8 (5-9)	7 (5-9)	7 (5-8)	0.05	0.03	5.0-7.5
Protein	1 (0-30)	1 (0-30)	1 (0-30)	0.61	0.31	Negative
Heme	0 (0-50)	10 (0-250)	0 (0-25)	0.01	0.14	Negative
Erythrocytes	0 (0-14)	1 (0-50)	0 (0-4)	0.66	0.26	0-5/HPF
Leukocytes	0 (0-17)	0 (0-9)	0 (0-3)	0.63	0.44	0-5/HPF
Casts	0 (0-0)	0 (0-1)	0 (0-0)	0.14	N/A	None
Epithelial cells	0 (0-2)	0 (0-5)	0 (0-2)	0.20	0.63	Variable
Crystals	0 (0-20)	0 (0-5)	0 (0-50)	0.72	0.44	None
Bacteria	0 (0-1)	0 (0-0)	0 (0-0)	0.37	> 0.99	None

**SUPPLEMENTARY TABLE 3.** Nutrient analysis including plasma amino acid and serum L-carnitine concentrations in dogs consuming meat-based diets (baseline) versus plant-based nutrition (6 and 12 months). Values refer to median (first quartile - third quartile). Reference values for first and third quartiles were provided by UCD. We requested reference intervals (minimum to maximum) from this laboratory, but were informed they were not available.

When evaluating the entire data set (as shown in Figure 2), some data points were below the  $Q_1$  for six of the ten *essential* AAs. We initially extrapolated the reference interval (minimum to maximum) for the essential amino acid tryptophan (11 – 103 nmol/L) based on the first ( $Q_1$ ) and third ( $Q_3$ ) quartiles, and the interquartile range (IQR), as follows. If  $IQR = Q_3 - Q_1$ , the maximum can be calculated as ( $Q_3 + 1.5 \times IQR = 68 + 1.5 \times 23 = 103$ ) and the minimum as ( $Q_1 - 1.5 \times IQR = 45 - 1.5 \times 23 = 11$ ). We moreover used extrapolation to calculate minimum values for arginine (28), leucine (36.5), methionine (15), threonine (28.5), and valine (56.5), as shown in Figure 2.

Three of the *essential* AA levels were significantly different between baseline and endpoint, including methionine ( $p = 0.02$ ), phenylalanine ( $p = 0.01$ ), and tryptophan ( $p = 0.01$ ). Values were either within or above the interquartile reference intervals (first quartile - third quartile) provided by UCD, and above the minimum values derived through extrapolation. The values for all three of these AAs trended upwards with higher values at endpoint compared to baseline.

Statistically significant differences were found in the levels of five *non-essential* AAs between baseline and endpoint, including alanine ( $p < 0.001$ ), cystathionine ( $p < 0.001$ ), glutamate ( $p = 0.02$ ), glutamine ( $p < 0.001$ ), serine ( $p = 0.03$ ), and tyrosine ( $p = 0.001$ ). All measured within or

above the reference intervals and values for all five AAs exhibited an upwards trend. Another four *non-essential* AAs (cysteine, hydroxyproline, ornithine, proline) measured below the first quartile, but showed no statistically significant differences between baseline and endpoint values. Cysteine levels were low, or below detection, in most samples (which is commonly attributed to storage loss). Hydroxyproline and proline levels increased over time, while ornithine levels decreased slightly.

Amino acid	Baseline (nmol/ml)	6 months (nmol/ml)	12 months (nmol/ml)	P-value (Friedman)	P-value (Wilcoxon)	Ref. Values (UC Davis)
<i>Essential</i>						
Arginine	118 (112-144)	103 (99-109)	127 (112-164)	< 0.001	0.72	85-123
Histidine	84 (79-89)	86 (84-88)	94 (88-105)	0.01	0.07	60-80
Isoleucine	84 (73-90)	64 (58-68)	75 (61-82)	0.001	0.22	40-57
Leucine	134 (114-154)	125 (111-129)	138 (127-179)	0.03	0.12	95-134
Lysine	149 (140-160)	156 (140-169)	167 (148-238)	0.13	0.09	94-159
Methionine	51 (48-56)	51 (49-53)	68 (51-78)	0.01	0.02	45-65
Phenylalanine	61 (59-64)	72 (66-73)	72 (66-79)	0.002	0.01	39-52
Threonine	172 (136-298)	162 (151-185)	226 (207-246)	0.03	0.60	138-211
Tryptophan	36 (24-42)	35 (34-42)	52 (39-75)	0.01	0.01	45-68
Valine	173 (137-201)	155 (145-158)	179 (157-214)	0.04	0.32	130-179
<i>Non-essential</i>						
Alanine	396 (352-432)	562 (551-588)	578 (533-651)	< 0.001	< 0.001	320-455
Asparagine	63 (50-73)	45 (42-49)	55 (47-96)	0.01	0.95	30-49
Aspartate	11 (9-13)	9 (6-9)	10 (9-15)	0.02	0.79	6-8
Butyrate	22 (18-24)	32 (25-34)	17 (15-27)	0.06	0.57	ND
Citrulline	52 (45-56)	36 (35-44)	53 (34-65)	0.04	0.92	27-50
Cystathionine	5 (5-6)	8 (7-9)	8 (8-10)	< 0.001	< 0.001	ND
Cysteine	0 (0-1)	0 (0-4)	1 (0-1)	0.32	0.34	36-53

Glutamate	72 (59-84)	97 (74-124)	91 (75-106)	0.01	0.02	15-26
Glutamine	564 (539-590)	643 (626-683)	735 (693-773)	< 0.001	< 0.001	417-569
Glycine	201 (185-243)	185 (174-187)	241 (195-351)	0.01	0.38	207-310
Hydroxyproline	21 (16-33)	12 (9-18)	29 (18-51)	0.01	0.22	44-78
Methylhistidine1	11 (9-14)	6 (4-9)	8 (3-13)	0.01	0.08	ND
Methylhistidine3	5 (4-7)	2 (1-4)	5 (3-6)	0.01	0.45	ND
Ornithine	19 (9-27)	14 (12-16)	15 (13-18)	0.62	0.19	23-43
Proline	161 (112-201)	147 (129-172)	202 (161-206)	0.08	0.15	174-304
Serine	121 (114-128)	121 (112-125)	137 (122-161)	0.01	0.03	87-126
Taurine	106 (79-132)	89 (78-92)	136 (115-152)	< 0.001	0.06	60-90
Tyrosine	34 (31-39)	44 (39-52)	53 (47-58)	< 0.001	0.001	30-47
L-Carnitine	19 (13-21)	27 (17-31)	35 (14-43)	0.09	0.06	ND

**SUPPLEMENTARY TABLE 4.** Nutrient analysis of serum vitamin concentrations in dogs

consuming meat-based diets (baseline) versus plant-based nutrition (6 and 12 months). Values refer to median (minimum - maximum).

Vitamin	Unit	Baseline	6 months	12 months	P-value (Friedman)	P-value (Wilcoxon)	Ref. Values (MSU/TAMU)
<i>Lipid-soluble</i>							
Vitamin A	ng/mL	734 (437-1,295)	924 (600-1,292)	979 (769-1,330)	0.01	0.01	400-1,200
Vitamin D	nmol/L	120 (57-418)	228 (91-383)	257 (173-418)	< 0.001	0.004	109-423
Vitamin E	ug/mL	42 (21-74)	31 (25-56)	37 (25-57)	0.62	0.33	4-12
<i>Water-soluble</i>							
Folate (B9)	ug/L	9 (5-23)	11 (4-15)	11 (4-37)	0.04	0.09	8 – 24
Cobalamin (B12)	ng/L	364 (261-639)	427 (310-619)	426 (278-505)	0.25	0.17	251 - 908

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