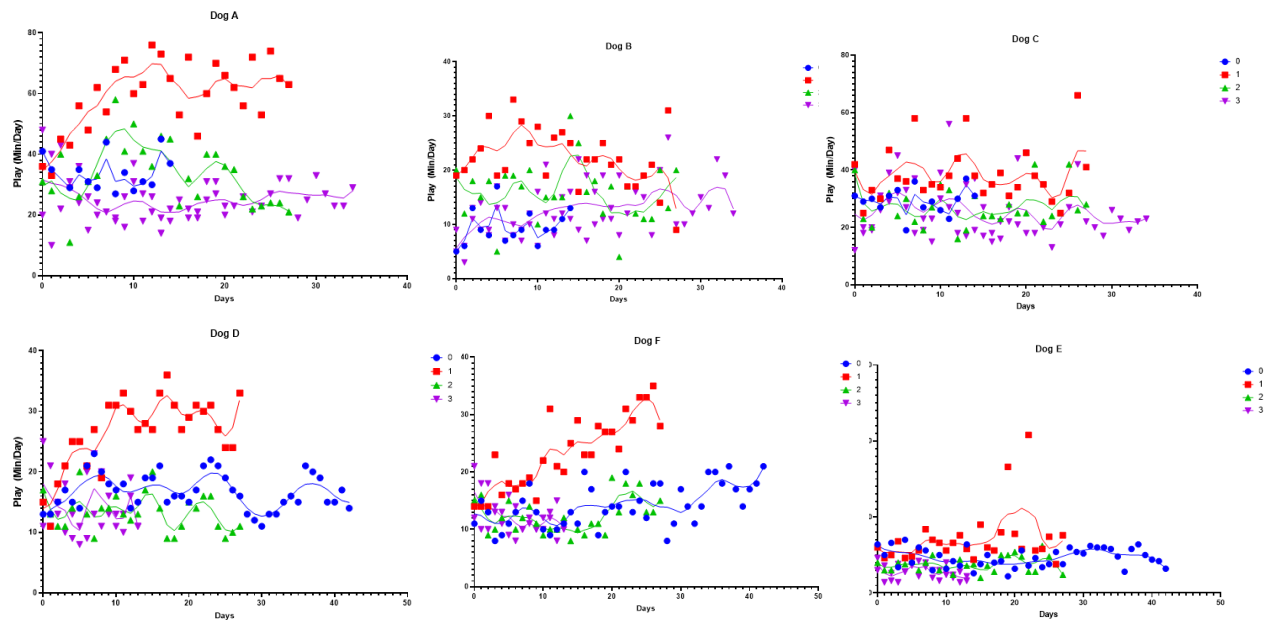


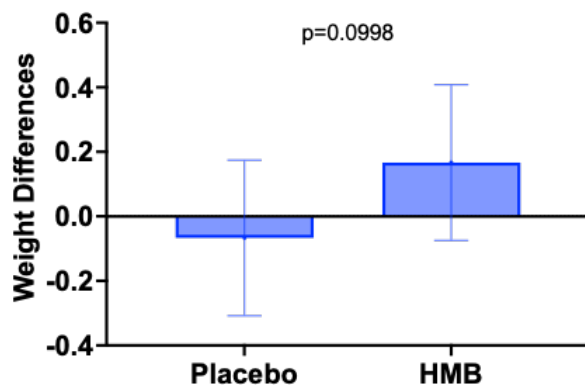
Supplemental Info:

Positive Response to HMB

No Response to HMB



Supplemental Figure 1: Individual dog exertional (play) activity data for each dosing regimen (HMB and placebo). Red square = HMB treatment. Blue circle = baseline measurement pre HMB treatment. Green triangle = placebo treatment. Purple upside down triangle = baseline measurement pre-placebo treatment.



Supplemental Figure 2: Weight difference showed a trend for increased body weight in the HMB-treated group. 2-way ANOVA performed between HMB and placebo.

Supplemental Table 1: Extracellular compartment pool size of amino acids

	Mean of Placebo (n=6)	Mean of HMB (n=6)	P value	n
Arginine	995.2 [748.8, 1242]	885.3 [678.2, 1092]	0.4930	40
Citrulline	70.33 [50.98, 89.68]	64.74 [45.92, 83.56]	0.6767	38
Glutamate	489.5 [289, 690]	668.6 [419.0, 918.2]	0.2621	42
Glutamine	10922 [9434, 12410]	11107 [9646, 12568]	0.8581	40
Glycine	2374 [2043, 2705]	1578 [1362, 1792]	0.0001	41
Hydroxyproline	576.8 [366.1, 787.5]	152.0 [95.38, 208.6]	0.0002	40
Histidine	1283 [1125, 1441]	1263 [1127, 1399]	0.8472	42
Isoleucine	830.2 [662.8, 997.6]	878.9 [692.9, 1065]	0.6949	42
Leucine	1480 [1123, 1797]	1249 [965.3, 1533]	0.3306	36
Lysine	3380 [2888, 3872]	3446 [2997, 3895]	0.8420	30
Methionine	687.9 [626.3, 749.5]	648.4 [600.5, 696.3]	0.3077	39
Methyl-Histidine	54.09 [37.87, 70.31]	56.36 [40.28, 72.44]	0.8414	33
Ornithine	173.7 [144.5, 202.8]	148.0 [125.0, 171.0]	0.1660	42
Phenylalanine	1114 [1015, 1213]	1061 [978.3, 1184]	0.6415	41
Proline	2481 [2122, 2840]	1792 [1539, 2045]	0.0021	42
Taurine	763.5 [578.8, 948.2]	672.2 [510.9, 833.5]	0.4540	42

Threonine	2315 [1843, 2787]	2144 [1705, 2583]	0.5935	41
Tryptophan	34.72 [27.34, 42.10]	33.14 [26.39, 39.89]	0.7502	42
Tyrosine	1250 [1131, 1369]	1134 [985.6, 1282]	0.2193	38
Valine	1004 [824.2, 1184]	1000 [824.6, 1175]	0.9744	42

Values were in $\mu\text{mol/h}$; predicted mean [95 % CI]. Ordinary two-way ANOVA performed on the predicted WBP after ANCOVA on the TTR decay with covariants group, centered weight, and (ln-t) time. Bolded text was for $p < 0.05$. P values were for group and interaction groups. Tukey post hoc testing between Placebo vs HMB ($\wedge$) (*/+/ $\wedge$ p < 0.05, **/++/ $\wedge$ p < 0.01, ***/+++/ $\wedge$ p < 0.001).

Supplemental Table 2: Intracellular Disposal of Amino Acids

	Mean of Placebo (n=6)	Mean of HMB (n=6)	P value	n
Arginine	87.75 [75.47, 100.0]	76.60 [65.99, 87.21]	0.1696	40
Citrulline	3.630 [0.5276, 6.732]	3.522 [0.4823, 6.562]	0.9600	38
Glutamate	201.9 [130.2, 273.6]	224.8 [150.6, 299.0]	0.6545	42
Glutamine	934.7 [593.9, 1275]	939.6 [601.9, 1277]	0.9836	40
Glycine	280.3 [247.7, 312.9]	208.6 [182.9, 234.3]	0.0008	41
Hydroxyproline	8.670 [2.760, 14.58]	3.2020 [1.042, 4.998]	0.0674	40
Histidine	71.39 [-135.4, 278.1]	74.95 [-143.5, 293.4]	0.9810	42
Isoleucine	54.12 [40.51, 67.73]	64.49 [48.03, 80.95]	0.3291	42
Leucine	357.8 [188.1, 527.5]	321.2 [70.4, 472.0]	0.7436	36
Lysine	180.5 [43.47, 317.5]	184.6 [46.10, 323.1]	0.9662	30
Methionine	226.4 [67.60, 385.2]	176.3 [79.11, 273.5]	0.5858	39
Methyl-Histidine	0.2771 [0.03861, 0.5156]	0.2887 [0.04030, 0.5371]	0.9459	33
Ornithine	11.24 [-9.682, 32.16]	9.419 [-7.693, 26.53]	0.8921	42
Phenylalanine	364.0 [-466.6, 1195]	331.2 [-476.8, 1139]	0.9546	41
Proline	211.6 [180.5, 242.7]	163.1 [138.6, 187.6]	0.0151	42

Taurine	63.49 [55.12, 71.86]	62.89 [54.28, 71.50]	0.9198	42
Threonine	72.52 [60.84, 84.20]	73.08 [61.17, 84.99]	0.9461	41
Tryptophan	2.465 [1.741, 3.189]	2.399 [1.689, 3.109]	0.8958	42
Tyrosine	795 [-1238, 2828]	736.0 [-2162, 3634]	0.9731	38
Valine	137.1 [106.4, 167.8]	124.3 [97.30, 151.3]	0.5298	42
Values were in $\mu\text{mol/h}$; predicted mean [95 % CI]. Ordinary two-way ANOVA performed on the predicted WBP after ANCOVA on the TTR decay with covariants group, centered weight, and (ln-t) time. Bolded text was for $p < 0.05$. P values were for group and interaction groups. Tukey post hoc testing between Placebo vs HMB (^) (*/+/^p < 0.05, **/++/^p < 0.01, ***/+++/^p < 0.001).				

Supplemental Table 3: Q1: Intracellular compartmental pool size of amino acids

	Mean of Placebo (n=6)	Mean of HMB (n=6)	P value	n
Arginine	5931 [4512, 7350]	5155 [3902, 6408]	0.4105	40
Citrulline	140.6 [-54.29, 335.5]	148.2 [-49.20, 345.6]	0.956	38
Glutamate	5762 [2777, 8747]	6256 [3176, 9336]	0.8162	42
Glutamine	45359 [17462, 73256]	45324 [17512, 73136]	0.9986	40
Glycine	17321 [13324, 21318]	13126 [9824, 16428]	0.1069	41
Hydroxyproline	2137 [1339, 2935]	796.1 [512.1, 1080]	0.0018	40
Histidine	1006 [-3119, 5131]	1024 [-3287, 5335]	0.9952	42
Isoleucine	3531 [1744, 5318]	4363 [2219, 6507]	0.5483	42
Leucine	11184 [3750, 8618]	10153 [3481, 16825]	0.8341	36
Lysine	4297 [-101.6, 8696]	4352 [-142.9, 88847]	0.9859	30
Methionine	12957 [-4044, 29958]	9833 [-1753, 21418]	0.7586	39
Methyl-Histidine	69.64 [32.23, 107.1]	72.57 [33.73, 111.4]	0.9129	33
Ornithine	224.2 [-371.0, 819.4]	190.6 [-308.0, 689.2]	0.9306	42
Phenylalanine	9748 [-24608, 44104]	9039 [-24009, 42087]	0.9761	41
Proline	13943 [9947, 17939]	10908 [7781, 14035]	0.2285	42
Tau	6636 [4878, 8394]	6666 [4873, 8459]	0.9808	42

Threonine	4752 [3261, 6243]	5042 [3510, 6574]	0.7847	41
Tryptophan	83.39 [47.42, 119.4]	82.26 [45.90, 118.6]	0.9645	42
Tyrosine	23391 [-113283, 160065]	22216 [-137833, 182265]	0.991	38
Valine	6749 [4245, 9253]	5996 [3779, 8213]	0.651	42

Values were in $\mu\text{mol/h}$; predicted mean [95 % CI]. Ordinary two-way ANOVA performed on the predicted whole body production (WBP) after ANCOVA on the TTR decay with covariants group, centered weight, and (ln-t) time. Bolded text was for $p < 0.05$. P values were for group and interaction groups. Tukey post hoc testing between placebo vs HMB (^) (*/+/^p < 0.05, **/+/^p < 0.01, ***/+++/^p < 0.001).

Supplemental Table 4: Flux of amino acids between intracellular and extracellular compartment

	Mean of Placebo (n=6)	Mean of HMB (n=6)	P value	n
Arginine	99.17 [84.83,, 113.5]	87.88 [74.69, 101.1]	0.2453	40
Citrulline	2.381 [1.051, 3.711]	2.381 [0.9883, 3.774]	>0.9999	38
Glutamate	72.93 [58.62, 87.24]	97.08 [77.90, 116.3]	0.0452	42
Glutamine	427.3 [353.8, 500.8]	431.9 [356.7, 507.1]	0.9298	40
Glycine	187.2 [170.5, 203.9]	126.6 [115.4, 137.8]	<0.0001	41
Hydroxyproline	48.37 [34.70, 62.04]	13.63 [10.33, 16.93]	<0.0001	40
Histidine	26.33 [-1.662, 54.32]	25.13 [0.6548, 49.61]	0.9482	42
Isoleucine	42.62 [34.15, 51.09]	46.78 [37.71,55.85]	0.4998	42
Leucine	104.6 [88.37, 120.8]	90.41 [76.19, 104.8]	0.1844	36
Lysine	115.6 [60.69, 170.5]	116.7 [62.23, 171.2]	0.9772	30
Methionine	21.94 [20.00, 23.88]	20.16 [18.45, 21.87]	0.1674	39
Methyl-Histidine	1.867 [0.6109, 3.123]	1.946 [0.6359, 3.256]	0.9302	33
Ornithine	4.534 [1.169, 7.899]	3.920 [0.9816, 6.858]	0.782	42
Phenylalanine	29.28 [23.82, 34.74]	28.93 [22.58, 35.28]	0.9329	41
Proline	149.4 [132.9, 185.9]	109.5 [97.58, 121.4]	0.0002	42

Taurine	72.49 [61.63, 83.35]	64.73 [55.43, 74.03]	0.2759	42
Threonine	130.4 [95.17, 165.6]	127.2 [93.53, 160.9]	0.8948	41
Tryptophan	2.382 [1.752, 3.012]	2.305 [1.698, 2.912]	0.8594	42
Tyrosine	43.28 [38.50, 50.08]	40.26 [31.07, 49.45]	0.5924	38
Valine	70.00 [61.24, 78.76]	68.30 [59.35, 77.25]	0.7847	42
Values were in $\mu\text{mol/h}$; predicted mean [95 % CI]. Ordinary two-way ANOVA performed on the predicted WBP after ANCOVA on the TTR decay with covariants group, centered weight, and (ln-t) time. Bolded text was for $p < 0.05$. P values were for group and interaction groups. Tukey post hoc testing between placebo vs HMB ($\wedge$) (*/+/$\wedge$p < 0.05, **/++/$\wedge\wedge$p < 0.01, ***/+++/$\wedge\wedge\wedge$p < 0.001).				

Supplemental Table 5: Biochemical analysis of HMB and placebo groups

	Mean of Placebo (n=6)	Mean of HMB (n=6)	P Value
Albumin (g/dL)	3.125 [3.096, 3.154]	3.183 [3.135, 3.232]	0.2555
Albumin:Globulin Ratio	0.9361 [0.9104, 0.9619]	0.9639 [0.9338, 0.9940]	0.4078
ALKP (U/L)	21.83 [18.78, 24.89]	17.44 [16.92, 17.97]	0.3737
ALT (U/L)	307.1 [296.5, 317.6]	294.3 [269.5, 319.1]	0.9212
Amylase (U/L)	527.8 [506.4, 549.2]	530.6 [500.0, 561.2]	0.7596
AST (U/L)	354.2 [295.7, 412.6]	369.7 [249.5, 489.8]	0.3607
Bilirubin - Total (mg/dL)	0.1722 [0.1459, 0.1986]	0.1694 [0.1490, 0.1899]	0.4956
BUN (mg/dL)	11.19 [8.193, 14.20]	10.89 [8.178, 13.60]	0.7924
Calcium (mg/dL)	10.01 [9.929, 10.10]	10.09 [9.960, 10.23]	0.5460
Chloride (mEq/L)	109.8 [109.0, 110.5]	109.7 [108.4, 110.9]	0.8342
Cholesterol (mg/dL)	267.4 [260.5, 274.3]	280.1 [275.8, 284.3]	0.4404
Creatine Kinase (U/L)	16691 [13416, 19967]	16919 [12168, 21670]	0.5593
Creatinine (mg/dL)	0.3972 [0.3567, 0.4377]	0.4000 [0.3753, 0.4247]	0.5488
GGT (U/L)	3.444 [3.205, 3.683]	3.000 [3.000, 3.000]	0.2279
GLDH (U/L)	6.667 [1.624, 11.71]	2.000 [2.000, 2.000]	0.2845
Globulins (g/dL)	3.342 [3.315, 3.368]	3.319 [3.267, 3.372]	>0.9999

Glucose(mg/dL)	108.3 [105.5, 111.1]	108.8 [105.3, 112.2]	0.8555
Phosphorus (mg/dL)	3.925 [3.444, 4.406]	4.008 [3.476, 4.540]	0.3438
Potassium (mEq/L)	4.217 [4.157, 4.276]	4.269 [4.217, 4.322]	0.2911
Sodium (mEq/L)	147.9 [147.0, 148.8]	147.7 [146.8, 148.7]	0.7169
Sodium:Potassium Ratio	35.32 [34.81, 35.83]	35.00 [34.14, 35.85]	0.356
No abnormalities were detected in either group. Values are expressed as mean, 95% CI. Statistics were done by a two-tailed unpaired t-test.			