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Non-targeted LC-MS metabolomics approach for metabolic profiling of plasma and urine from pigs fed branched chain amino acids for maximum growth performance

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Table S1. Analyzed composition of experimental diets with different Ile content (g/kg, as-fed)¹.

Item	SID ² Ile:Lys					
	0.42	0.46	0.50	0.54	0.58	0.62
Crude protein (N × 6.25)	163.9	171.2	168.8	168.6	166.4	168.6
Alanine	5.6	5.8	5.7	5.7	5.6	5.6
Arginine	8.2	8.5	8.3	8.3	8.1	8.2
Aspartate	11.0	11.1	11.3	11.3	10.9	11.0
Cystine	2.7	2.7	2.7	2.7	2.7	2.7
Glutamate	34.4	34.6	34.0	33.5	32.6	32.4
Glycine	5.7	5.9	5.8	5.8	5.7	5.7
Histidine	4.4	4.5	4.5	4.5	4.4	4.4
Isoleucine	5.6	6.2	6.4	6.9	7.1	7.7
Leucine	13.3	13.5	13.1	13.1	12.7	12.8
Lysine	12.2	12.8	12.4	12.5	11.7	12.2
Methionine	4.3	4.7	4.5	4.6	4.3	4.4
Methionine + Cystine	7.0	7.4	7.2	7.2	7.0	7.0
Phenylalanine	8.5	8.8	8.7	8.7	8.4	8.5
Proline	10.5	10.6	10.6	10.6	10.6	10.6
Serine	6.9	7.1	7.1	7.1	6.9	7.0
Threonine	7.9	8.3	8.1	8.2	7.9	7.9
Valine	9.8	10.1	9.7	9.8	9.4	9.5

¹ Three samples of each diet were analyzed.

² SID = standardized ileal digestible.

Table S2. Analyzed composition of experimental diets with different Val content (g/kg, as-fed)¹.

Item	SID Val:Lys ²					
	0.58	0.62	0.66	0.70	0.74	0.78
Crude protein (N × 6.25)	178	181	182	179	180	180
Lys	11.8	12.2	12.2	12.0	12.0	12.1
Met	4.2	4.3	4.3	4.3	4.2	4.3
Met + Cys	7.3	7.4	7.3	7.3	7.2	7.3
Ile	7.1	7.2	7.2	7.1	7.1	7.2
Leu	12.9	13.1	13.0	12.9	12.9	13.0
Val	7.4	7.9	8.3	8.7	9.1	9.6
Thr	8.2	8.3	8.2	8.2	8.2	8.2
Phe	8.4	8.5	8.5	8.4	8.4	8.5
His	4.4	4.5	4.5	4.4	4.4	4.4
Ala	6.7	6.7	6.7	6.7	6.6	6.7
Arg	9.9	9.9	10.0	9.9	9.8	9.9
Asp	13.7	13.9	13.9	13.7	13.7	13.8
Glu	39.4	39.3	38.8	38.1	37.7	37.5
Gly	6.7	6.8	6.8	6.7	6.7	6.8
Pro	11.5	11.6	11.6	11.5	11.5	11.6
Ser	8.2	8.3	8.3	8.2	8.2	8.3

¹ Three samples of each diet were analyzed.

² SID = standardized ileal digestible.

Table S3. Analyzed composition of experimental diets with different Leu content (g/kg, as-fed)¹.

Item	SID ² Leu:Lys					
	0.70	0.80	0.90	1.00	1.10	1.20
Crude protein (N × 6.25)	157.3	155.8	153.8	158.3	155.1	158.5
Lys	11.77	11.77	10.72	11.89	11.36	11.60
Met	4.51	4.48	4.29	4.57	4.46	4.52
Met + Cys	7.20	7.17	6.98	7.28	7.14	7.22
Thr	7.90	8.00	7.73	8.07	7.90	7.99
Ile	7.01	7.06	6.80	7.07	6.95	7.11
Leu	8.87	9.93	10.72	12.28	13.01	14.27
Val	9.25	9.33	9.03	9.47	9.21	9.34
Phe	9.28	9.35	9.09	9.37	9.28	9.37
His	4.36	4.36	4.18	4.39	4.29	4.39
Ala	5.03	5.06	4.98	5.08	5.01	5.11
Arg	7.17	7.24	7.09	7.29	7.18	7.35
Asp	9.39	9.44	9.18	9.58	9.45	9.63
Glu	35.17	34.41	33.06	32.40	31.04	30.46
Gly	5.22	5.24	5.17	5.28	5.20	5.29
Pro	10.03	10.08	10.15	10.10	10.08	10.17
Ser	5.90	5.94	5.86	6.00	5.94	6.02

¹ Three samples of each diet were analyzed.

² SID = standardized ileal digestible.

Table S4. Effect of standardized ileal digestible (SID) Ile:Lys supply on performance of the pigs¹.

Item	SID Ile:Lys						<i>P</i> -value ³		
	0.42	0.46	0.50	0.54	0.58	0.62	SEM ²	Linear	Quadratic
Initial BW, kg	8.72	8.54	8.56	8.61	8.58	8.46	0.38	0.25	0.89
Final BW, kg	14.15	14.46	15.17	15.02	14.79	14.68	0.32	0.08	0.007
ADFI d 0-14, g	552	595	656	651	618	597	30	0.11	0.001
ADG d 0-14, g	398	420	470	459	443	435	22	0.08	0.007
FCR d 0-14	1.41	1.40	1.40	1.42	1.38	1.38	0.03	0.50	0.62

¹ Data represents the least square means of 16 pigs per treatment.

² Standard error of mean.

³ Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing standardized ileal digestible Ile to Lys.

Table S5. Effect of the standardized ileal digestible (SID) Val:Lys supply on performance of pigs¹.

Item	SID Val:Lys						SEM ²	P-value ³	
	0.58	0.62	0.66	0.70	0.74	0.78		Linear	Quadratic
Initial BW, kg	8.40	8.41	8.50	8.41	8.36	8.42	0.32	-	-
Final BW, kg	12.67	13.08	14.00	14.33	13.70	14.41	0.48	0.002	0.16
ADFI d 0-14, g	444	483	539	566	513	573	22	<0.001	0.09
ADG d 0-14, g	304	334	399	423	378	428	25	<0.001	0.06
G:F d 0-14, g/g	0.68	0.72	0.74	0.75	0.75	0.75	0.02	0.002	0.07

¹ 16 pigs were used for each treatment. The least square means are presented. Superscripts indicate differences among diets ($P < 0.05$).

² Standard error of mean.

³ Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of SID Val:Lys.

Online Supporting Information

Table S6. Effect of the standardized ileal digestible (SID) Leu:Lys supply on performance of the pigs¹.

Item	SID Leu:Lys						SEM ²	P-value ³	
	0.70	0.80	0.90	1.00	1.10	1.20		Linear	Quadratic
Initial BW, kg	7.77	7.69	7.78	7.79	7.79	7.82	0.68	-	-
Final BW, kg	10.76	11.74	12.11	12.17	12.18	12.27	0.32	0.001	0.02
ADFI d 0-14, g/d	359	399	456	456	454	479	25	<0.001	0.13
ADG d 0-14, g/d	212	281	308	312	313	320	23	<0.001	0.02
G:F d 0-14, g/g	0.60	0.72	0.69	0.69	0.69	0.66	0.03	0.29	<0.001

¹ 16 pigs were used for each treatment. The least squares means are presented. Within a row, means without a common superscript differ ($P < 0.05$).

² Standard error of mean.

³ Orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of increasing ratios of standardized ileal digestible Leu:Lys.

Online Supporting Information

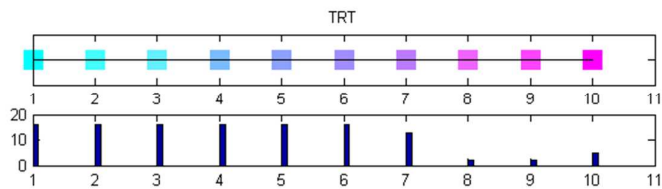
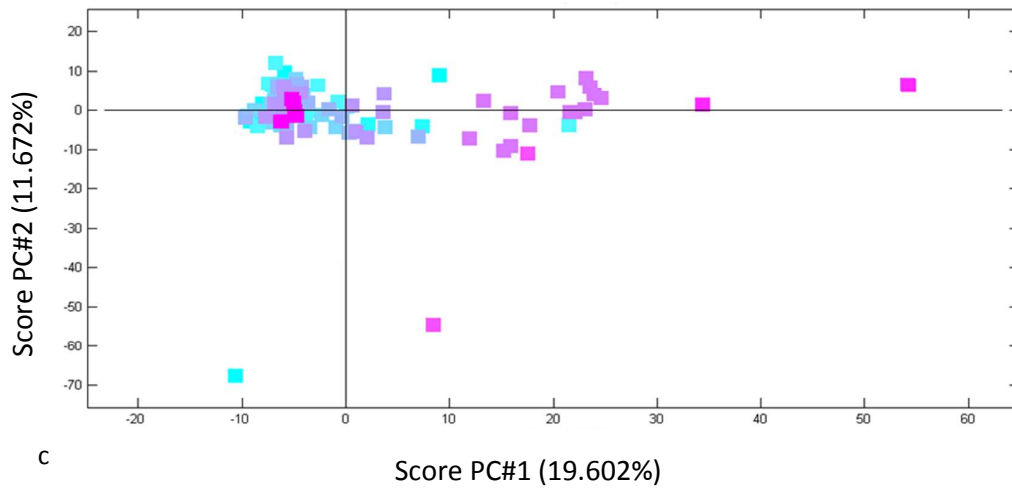
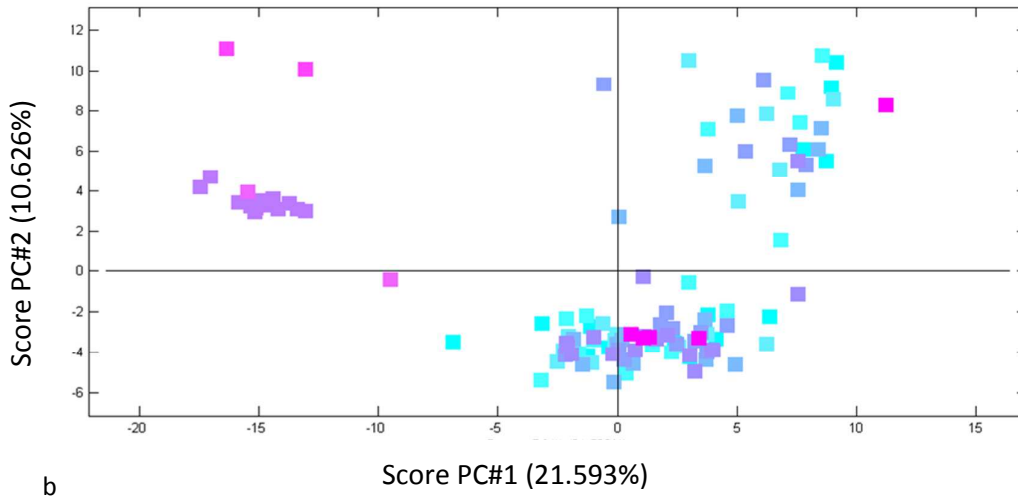
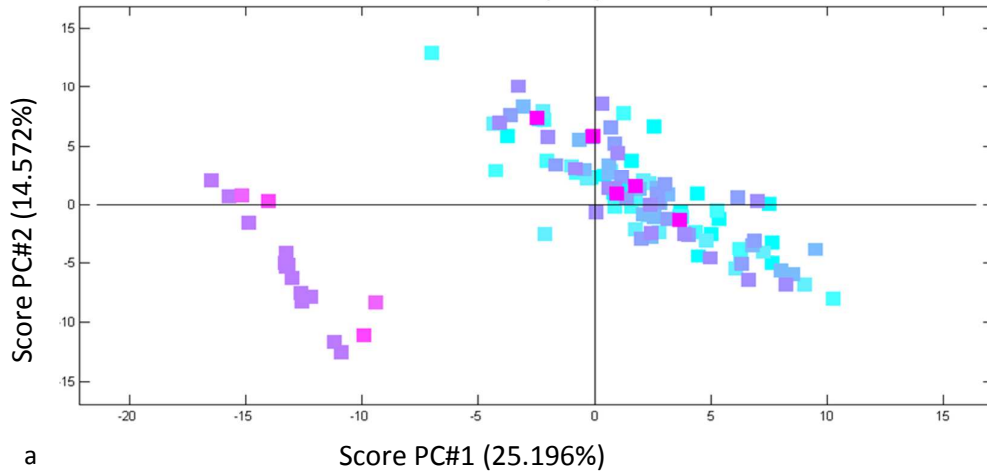
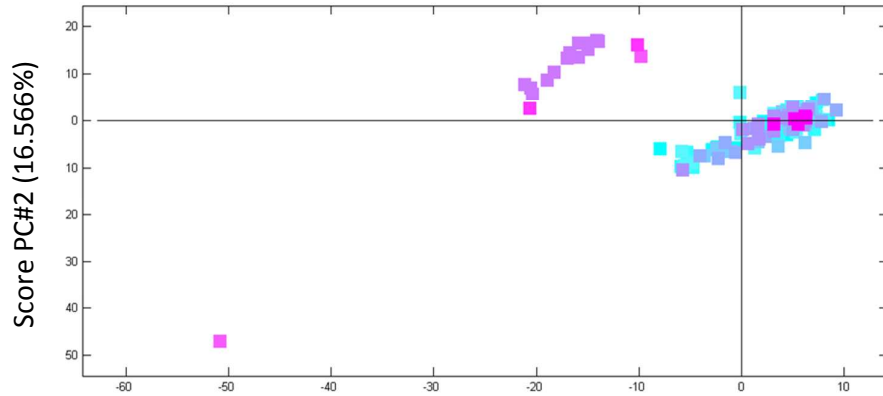
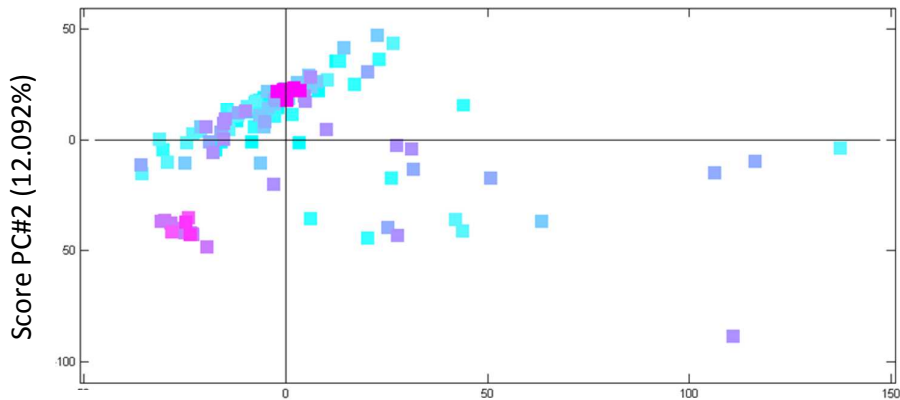


Figure S1. PCA score plots of groupings of all blood samples, blanks and quality controls from Ile (a), Val (b), and Leu (c) studies. The 10 color codes for treatments (TRT) corresponds to 6 dietary level of each BCAA in the Ile, Val, and Leu studies; 7 is blank including internal standards, 8 and 9 are blanks, and 10 is quality control (a pooled sample of all blood samples). The 6 TRT were 0.42, 0.46, 0.50, 0.54, 0.58, and 0.62 SID Ile:Lys, 0.58, 0.62, 0.66, 0.70, 0.74, and 0.78 SID Val:Lys, and 0.70, 0.80, 0.90, 1.00, 1.10, and 1.20 of SID Leu:Lys in Ile, Val, and Leu dose-response studies, respectively.

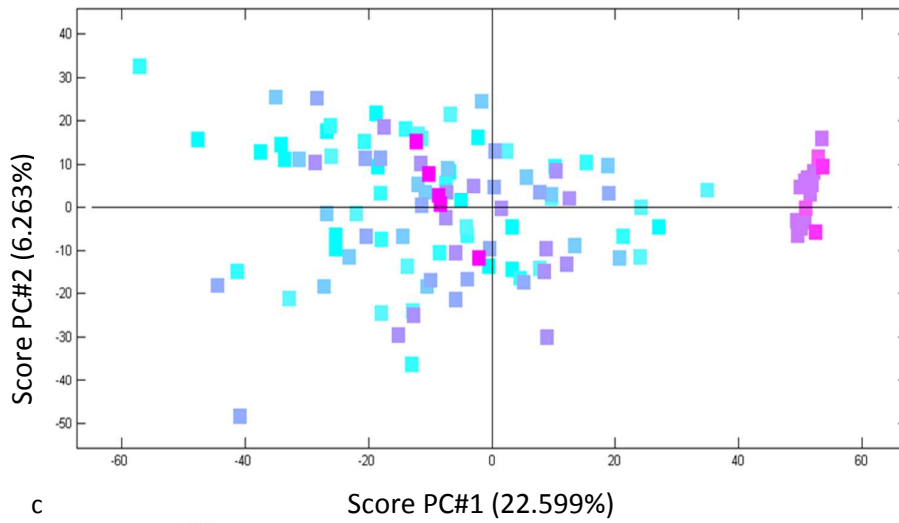
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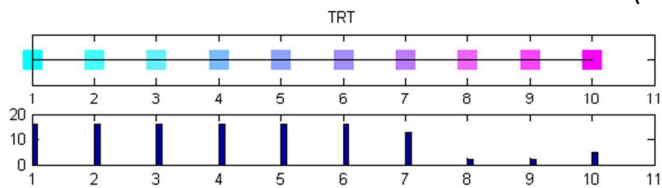
a



b



c



Online Supporting Information

Figure S2. PCA score plots of groupings of all urine samples, blanks and quality controls from Ile (a), Val (b), and Leu (c) studies. The 10 color codes for treatments (TRT) corresponds to 6 dietary level of each BCAA in the Ile, Val, and Leu studies; 7 is blank including internal standards, 8 and 9 are blanks, and 10 is quality control (a pooled sample of all urine samples). The 6 TRT were 0.42, 0.46, 0.50, 0.54, 0.58, and 0.62 SID Ile:Lys, 0.58, 0.62, 0.66, 0.70, 0.74, and 0.78 SID Val:Lys, and 0.70, 0.80, 0.90, 1.00, 1.10, and 1.20 of SID Leu:Lys in Ile, Val, and Leu dose-response studies, respectively.