

Supplementary Materials:

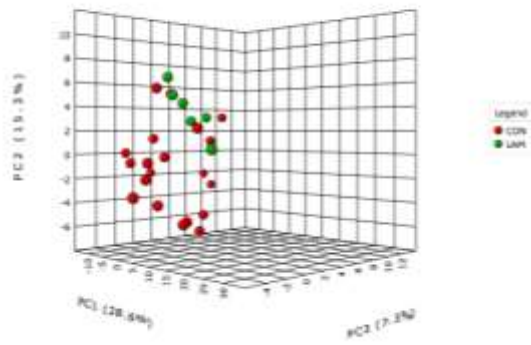
Table S1. Concentrations of altered urine metabolites [mean (SD)] in lame and healthy control cows at -8 weeks before parturition.

Metabolite μM	Lameness	Control	P-value	fold
1,3-Dimethylurate	7.02 (2.57)	4.79 (2.52)	0.05	1.47
2-Aminobutyrate	11.64 (2.39)	21.27(8.44)	0.001	-1.83
3-Hydroxy-3-methylglutarate	5.07 (1.52)	9.75 (3.71)	0.001	-1.92
3-Indoxylsulfate	41.21 (16.45)	77.50 (48.89)	0.03	-1.88
Acetoacetate	10.2 (2.29)	17.63 (11.27)	0.03	-1.73
Adipate	63.76 (15.91)	87.58 (24.09)	0.03	-1.37
Alanine	21.33 (3.50)	33.75 (17.81)	0.04	-1.58
Ascorbate	19.91 (7.27)	11.88 (6.33)	0.01	1.68
Ethanol	17.70 (5.37)	36.10 (25.66)	0.04	-2.04
Formate	24.63 (6.72)	49.64 (29.68)	0.01	-2.02
Hypoxanthine	2.90 (0.92)	1.77 (1.12)	0.03	1.64
Leucine	9.56 (3.91)	5.49 (2.68)	0.01	1.74
Lysine	30.59 (3.77)	18.44 (7.77)	0.001	1.66
Methylmalonate	11.98 (3.36)	17.21 (5.18)	0.03	-1.44
N,N-Dimethylglycine	1.79 (0.79)	4.07 (1.92)	0.01	-2.27
N-Acetylglutamate	9.91 (1.53)	13.58 (4.46)	0.02	-1.37
N-Acetylglutamine	9.49 (1.70)	16.60 (9.10)	0.01	-1.75
O-Phosphocholine	3.10 (1.64)	1.44 (1.41)	0.03	2.16
Pantothenate	13.12 (3.32)	9.58 (3.47)	0.03	1.37
Propylene glycol	10.49 (1.92)	16.94 (6.62)	0.01	-1.61
Salicylate	18.11 (5.84)	35.46 (24.39)	0.03	-1.96
Serine	72.78 (23.73)	115.39 (43.44)	0.031	-1.59
Trimethylamine	1.10 (0.28)	1.99 (0.88)	0.001	-1.81
Tyrosine	8.90 (2.76)	15.40 (5.47)	0.01	-1.73
Valine	6.96 (1.77)	11.72 (3.77)	0.01	-1.68
Uracil	15.66 (5.98)	146.54 (99.05)	0.0001	-9.36
Xylose	22.58 (8.66)	10.72 (7.82)	0.01	2.11

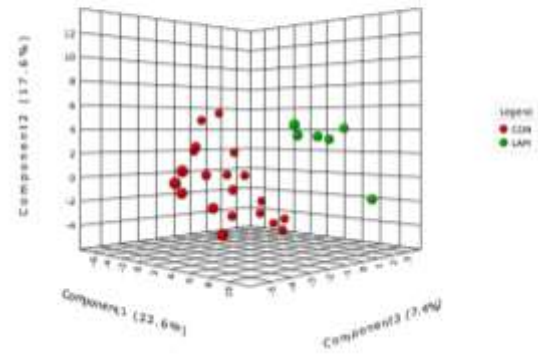
Table S2. Concentrations of altered urine metabolites [mean (SD)] in lame and healthy control cows at -4 weeks before parturition.

Metabolite μM	Lameness	Control	p-value	Fold Change
2-Hydroxyvalerate	91.54 (26.73)	67.67 (19.98)	0.02	1.35
3-Aminoisobutyrate	18.89 (5.51)	13.07 (4.5)	0.01	1.45
Acetylsalicylate	19.15 (8.87)	12.29 (6.52)	0.04	1.56
Alloisoleucine	13.80 (5.33)	7.82 (5.20)	0.01	1.77
Aspartate	16.00 (6.37)	9.79 (5.00)	0.01	1.64
Formate	27.88 (22.8)	42.67(23.83)	0.03	-1.53
Glucose	23.40 (6.91)	14.56 (9.22)	0.01	1.61
Histidine	29.00 (12.50)	15.35 (5.04)	0.04	1.89
Hypoxanthine	4.61 (3.49)	1.73 (1.37)	0.003	2.67
Isocitrate	50.10 (17.90)	35.16 (14.13)	0.02	1.42
Lysine	32.28 (16.54)	15.84 (6.79)	0.001	2.04
Methanol	18.36 (14.38)	6.80 (3.56)	0.01	2.7
N,N-Dimethylglycine	1.81 (0.95)	4.01 (3.17)	0.01	-2.21
N-Acetylaspartate	19.71 (10.73)	8.50 (4.42)	0.05	2.32
O-Phosphocholine	1.77 (1.24)	1.25 (2.30)	0.03	1.41
Pantothenate	12.89 (5.47)	6.81 (3.04)	0.001	1.89
Threonine	18.01 (5.50)	10.02 (8.13)	0.001	1.8
Tiglylglycine	24.94 (12.01)	15.26 (7.72)	0.02	1.63
Tryptophan	26.20 (12.20)	15.56 (8.02)	0.01	1.68
Tyrosine	6.90 (4.36)	13.06 (5.05)	0.01	-1.89
Urea	976.13 (241.87)	6035.98 (4370.96)	0.01	-6.18
Uracil	29.96 (9.26)	144.30 (96.80)	0.01	-4.82
Xylose	18.31 (6.23)	9.16 (5.17)	0.002	2
trans-Aconitate	16.76 (3.74)	7.70 (4.56)	0.001	2.18
beta-Alanine	9.66 (6.51)	4.81 (2.64)	0.01	2.01
pi-Methylhistidine	19.16 (8.96)	12.46 (6.25)	0.04	1.54
t-Methylhistidine	27.76 (9.44)	13.36 (6.27)	0.001	2.08

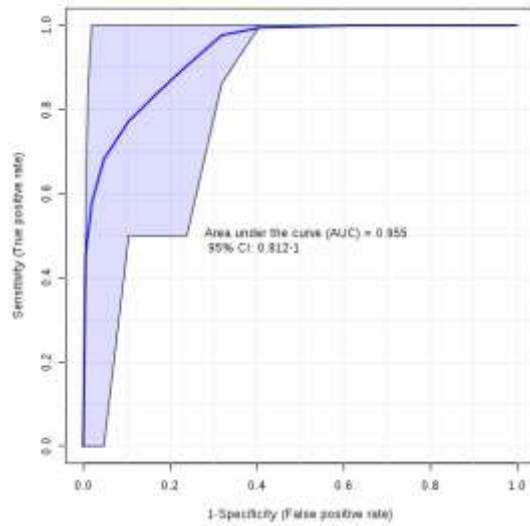
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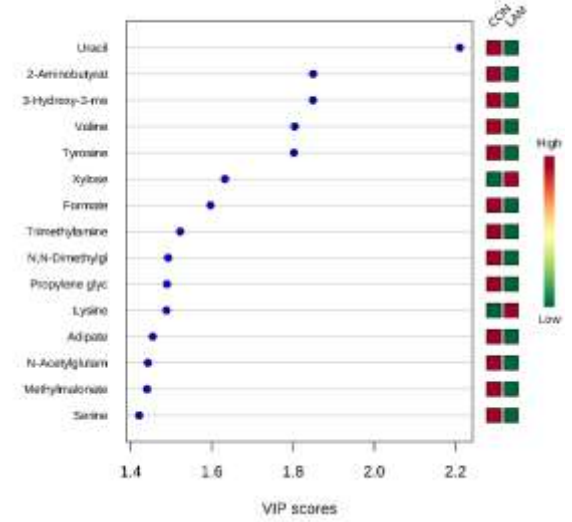
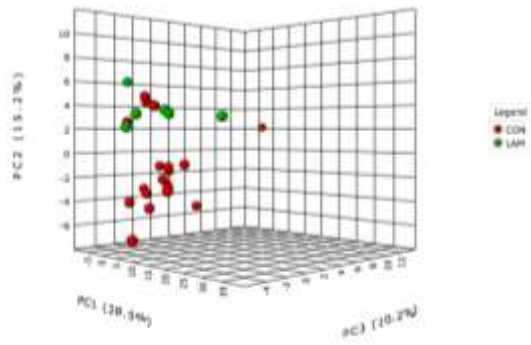
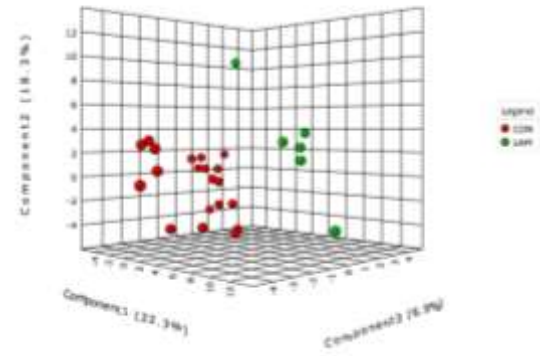


Figure S1. Visualization of (A) principal component analysis (PCA), and (B) partial least squares-discriminant analysis (PLS-DA) of 20 CON and 6 LAM (lame) cows 8 weeks before lameness diagnosis, and (C) ROC curve and (D) variables ranked by variable importance in projection (VIP).

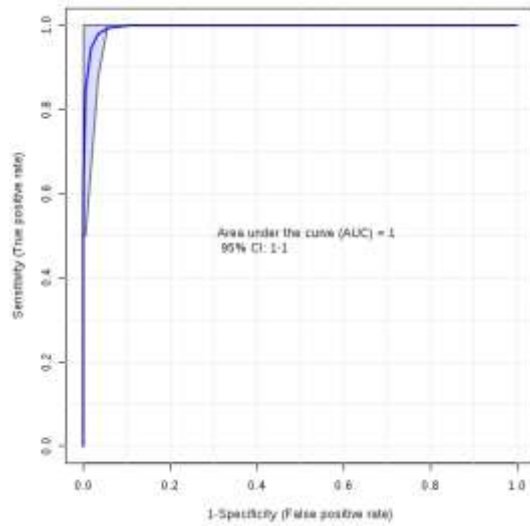
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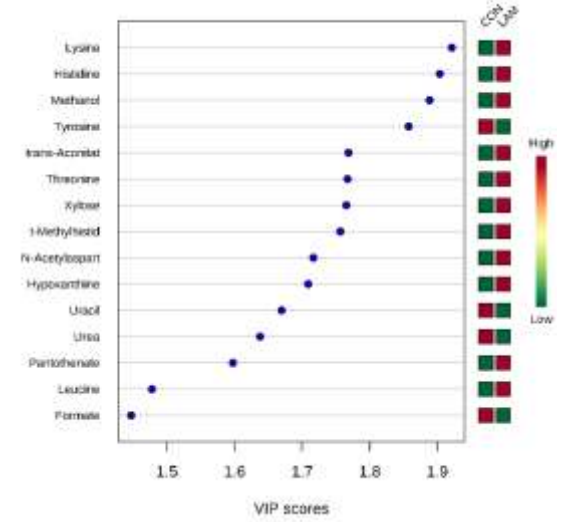
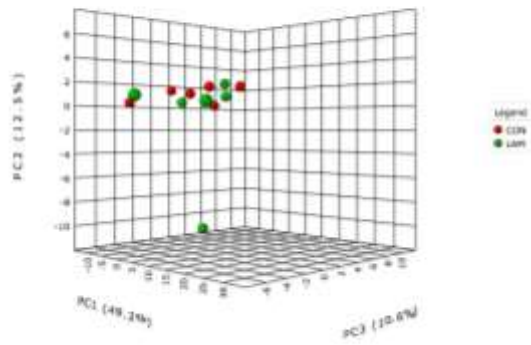
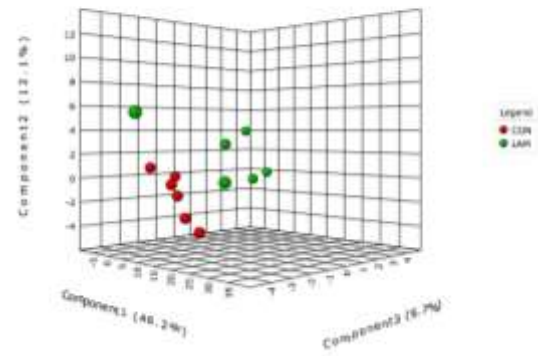


Figure S2. Visualization of (A) principal component analysis (PCA), and (B) partial least squares-discriminant analysis (PLS-DA) of 20 CON and 6 LAM (lame) cows 4 weeks before lameness diagnosis, and (C) ROC curve and (D) variables ranked by variable importance in projection (VIP).

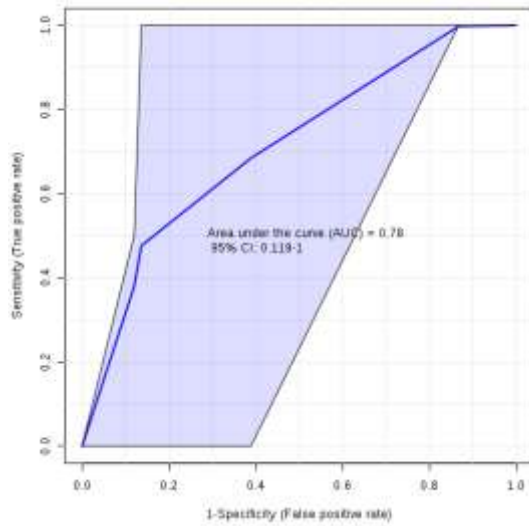
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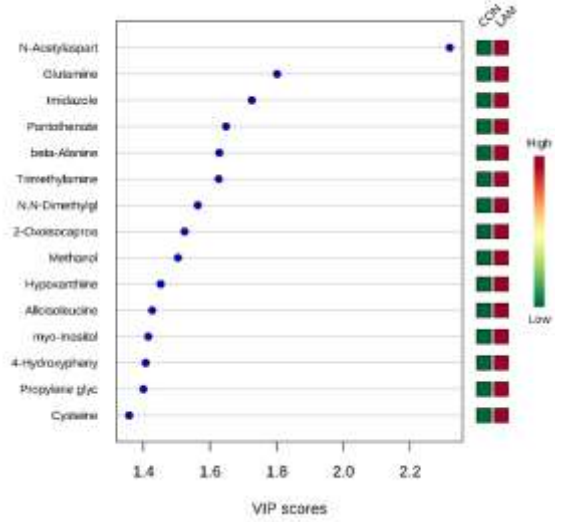
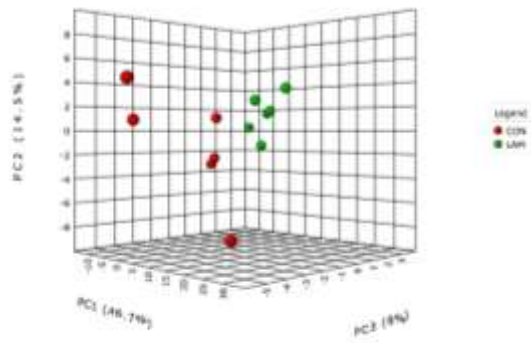
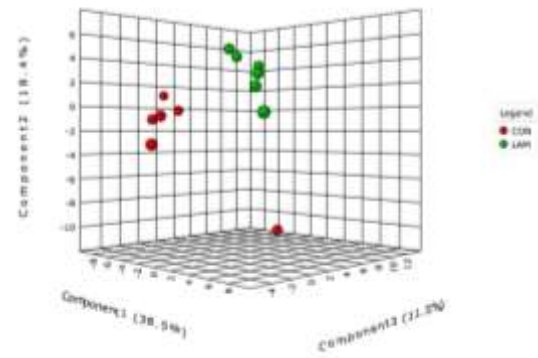


Figure S3. Visualization of (A) principal component analysis (PCA), and (B) partial least squares-discriminant analysis (PLS-DA) of 20 CON and 6 LAM (lame) cows 4 weeks after lameness diagnosis, and (C) ROC curve and (D) variables ranked by variable importance in projection (VIP).

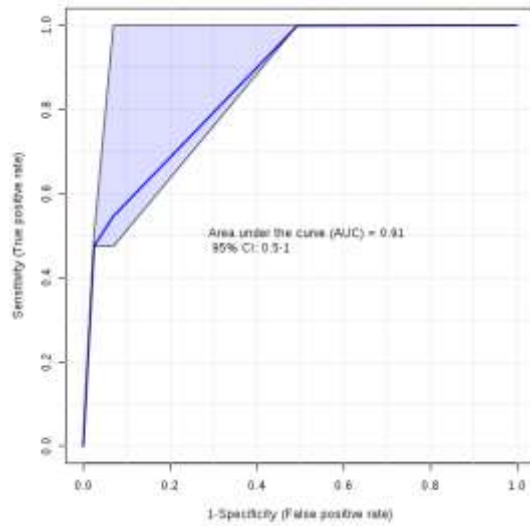
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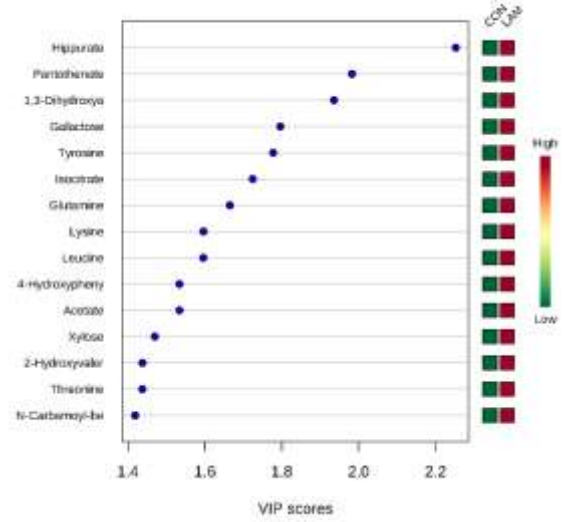


Figure S4. Visualization of (A) principal component analysis (PCA), and (B) partial least squares-discriminant analysis (PLS-DA) of 20 CON and 6 LAM (lame) cows 8 weeks after lameness diagnosis, and (C) ROC curve and (D) variables ranked by variable importance in projection (VIP).