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Systemic Metabolic Plasticity in Equines: Comparative Metabolomics of High-Performance and Indigenous Breeds Under Real-World Management

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Article

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Abstract

Systemic metabolic plasticity in agricultural and mammalian models is profoundly shaped by the interaction of genetic background, dietary composition, and rearing environment. However, comparative baseline metabolic phenotypes between high-performance breeds and indigenous animals under distinct real-world management remain limited. We comprehensively characterized the baseline plasma metabolomes of Thoroughbred horses (Seoul Racecourse) and Jeju Native Horses (Jeju Racecourse) using a rigorously validated untargeted ultra-high-performance liquid chromatography-Orbitrap mass spectrometry (UHPLC-Q-Exactive MS) platform. Multivariate statistical analyses revealed distinct plasma metabolic profiles between the two populations, largely associated with their distinct breed characteristics, regional environments, and husbandry practices—specifically, concentrate-based intensive feeding in Thoroughbreds versus forage-oriented management in Jeju Native Horses. Pathway enrichment highlighted prominent alterations involving nitrogen and amino acid metabolism, tricarboxylic acid (TCA) cycle-associated energy metabolism (e.g., citrate and malate), and folate-dependent one-carbon metabolism. Profound reprogramming in one-carbon networks further highlighted the interconnected roles of methionine, serine, and glycine in methyl-group availability and cellular redox homeostasis. While environmental and demographic variables are inherently intertwined in real-world field cohorts, this reproducible metabolic separation successfully captures the composite phenotypic divergence, providing a robust analytical baseline framework for comparative equine metabolomics. Overall, these findings demonstrate substantial differences in systemic metabolic phenotypes between the two breeds, providing broader biochemical insights into metabolic plasticity and adaptation in mammals exposed to divergent real-world nutritional and environmental management.

Introduction

Systemic metabolic homeostasis in large agricultural herbivores is profoundly shaped by the complex interaction between genetic background, dietary composition, and rearing environment [1, 2]. Understanding how specific breeds adapt metabolically to localized nutritional management is crucial for optimizing feed utilization, animal welfare, and overall livestock husbandry practices [3]. In particular, examining how the shift from evolutionary forage-based diets to intensive concentrate-based feeding alters baseline metabolic phenotypes provides foundational insights into the nutritional biochemistry and metabolic plasticity of agricultural animals [4, 5].

Equines represent an exceptional biological model for investigating this metabolic plasticity, as they are highly sensitive hindgut fermenters [3, 4]. While these animals have evolved to utilize structural carbohydrates via microbial fermentation in the hindgut, modern intensive animal husbandry frequently relies on starch-rich, concentrate-heavy diets to meet high physiological output demands [5–7]. Such differences in nutritional management may influence systemic amino acid availability, nitrogen handling, and energy metabolism in horses [5, 7].

In South Korea, two distinct equine populations provide a natural comparative setting to study these gene-environment-diet interactions [8, 9]. Thoroughbreds managed at inland facilities such as Seoul represent a selectively bred population maintained for high athletic performance, maintained under intensive husbandry with concentrate-centric feeding regimens and limited pasture access [7, 10]. In stark contrast, Jeju Native Horses are a hardy, indigenous breed with genetic signatures associated with adaptation to the local environment of Jeju Island, managed through traditional, forage-oriented grazing systems on Jeju Island [11, 12]. While previous studies have documented their genetic divergences [8, 9, 11], the extent to which contrasting breed backgrounds and real-world rearing and feeding environments jointly shape their baseline plasma metabolomes remains poorly understood.

To characterize these complex host-diet metabolic interactions, untargeted metabolomics provides a top-down systems biology approach [13]. Recent advancements in high-resolution ultra-high-performance liquid chromatography coupled with Orbitrap mass spectrometry (UHPLC-Q-Exactive MS), combined with robust data deconvolution algorithms such as MS-DIAL [14–17], enable comprehensive profiling of circulating metabolites [10]. This high-resolution analytical workflow enables characterization of circulating metabolic differences associated with contrasting nutritional and environmental conditions, facilitating the discovery of reliable biochemical signatures associated with environmental and nutritional adaptation [10, 13].

Therefore, this study aimed to comprehensively profile the baseline plasma metabolomes of Thoroughbreds (Seoul) and Jeju Native Horses (Jeju) using a rigorously validated untargeted UHPLC-Q-Exactive MS workflow. We hypothesized that the divergent nutritional regimens—concentrate- versus forage-centric diets—coupled with distinct genetic backgrounds would be associated with differences in central systemic metabolic networks, particularly targeting amino acid utilization, nitrogen metabolism, and tricarboxylic acid (TCA) cycle-associated energy homeostasis [5]. Ultimately, by characterizing these biochemical differences, this study seeks to identify metabolic pathways associated with contrasting nutritional and environmental management in equines, providing novel insights into the metabolic plasticity of large herbivores in agricultural research [5, 13].

Materials and methods

Materials

Phenacetin (used as an internal standard, ISTD), ammonium formate (analytical grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile (ACN) and deionized water (DW) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Formic acid (FA, analytical grade) was obtained from Junsei Chemical Co. (Tokyo, Japan). All solvents and reagents were used without further purification. The ISTD working solution was prepared by dissolving phenacetin in ACN at a concentration of 100 ng/mL.

Study animals and environmental conditions

A total of 99 horses were included in this study, comprising 49 Thoroughbred horses from Seoul Racecourse and 50 Jeju Native Horses from Jeju Racecourse, South Korea (Fig. 1). The Seoul group represented an inland population maintained primarily for performance-related purposes under a temperate climate. These horses were maintained under a concentrate-based feeding regimen with limited access to pasture. In contrast, the Jeju group consisted of horses reared under a maritime climate and managed using traditional husbandry practices, including a forage-based diet and access to natural pasture. The mean age of the horses was 4.3 ± 1.2 years in the Seoul group and 6.1 ± 2.4 years in the Jeju group. The sex distribution (male: female: gelding) was 18:23:8 for the Seoul group and 10:12:28 for the Jeju group. The samples were collected between June and July 2024. All animals were clinically healthy, maintained under standard veterinary care, and had not received any medications for at least two months prior to sample collection.

Sample collection

Blood samples were collected via jugular venipuncture into heparinized vacutainer tubes (BD Vacutainer®, Becton-Dickinson, Franklin Lakes, NJ, USA) under resting and non-exercised conditions. At the time of sampling, none of the horses had undergone intense physical activity. Only light warm-up routines, such as paddock walking or short-distance track movement, were permitted, and samples were collected in the early morning under resting conditions. The samples were processed within one hour of collection by centrifugation at 3,000 rpm for 10 minutes to isolate plasma. Aliquots were stored at −20°C until further metabolomic analysis. The Korea Racing Authority Institutional Animal Care and Use Committee (IACUC) determined that this study was exempt from IACUC review and approval because it used only residual plasma samples originally collected during routine equine doping control activities. No additional animals were used, no additional biological specimens were collected, and no animal procedures or interventions were performed for this study. No euthanasia or anesthesia was performed because the study involved only blood collection from live horses. Blood sampling was conducted using a minimally invasive procedure that did not cause substantial pain or distress to the animals. Therefore, anesthesia was not required for sample collection.

All procedures were performed in accordance with the relevant institutional, national, and international guidelines and regulations for the care and use of animals.

Plasma sample preparation and UHPLC–Q-Exactive MS analysis

Plasma aliquots (100 µL) were deproteinized by the addition of 300 µL of ACN containing phenacetin (100 ng/mL) as an ISTD. Following brief vortex mixing, the samples were centrifuged at 13,200 rpm for 5 min. The resulting supernatants were transferred to LC vials, and a 10 µL volume was injected into a Thermo UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA). Chromatographic separation was

achieved using a Luna Omega C18 column (1.6 μm, 2.1 $\times$ 100 mm; Phenomenex, Torrance, CA, USA). The mobile phase was composed of 5 mM ammonium formate in water (pH 3.0, solvent A) and 0.1% formic acid in ACN (solvent B). The gradient elution program was programmed as follows: 10% B from 0 to 5 min, linearly increased to 100% B from 5 to 23 min, held briefly, returned to 10% B at 23.1 min, and followed by column re-equilibration until 25 min. The flow rate was maintained at 0.25 mL/min, the column temperature was set to 40°C, and the autosampler was kept at 4°C throughout the analytical run. Mass spectrometric detection was performed on a Q-Exactive HF-X hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) equipped with an electrospray ionization (ESI) source operated in positive ionization mode. The optimized instrumental parameters were established as follows: heater temperature, 425°C; capillary temperature, 320°C; sheath gas flow rate, 50 arb; auxiliary gas flow rate, 10 arb; and ionization voltage, 3.5 kV. Data acquisition was executed using a data-dependent acquisition (DDA) mode with a loop count TopN of 25 across a mass range of *m/z* 100–1500. The normalized collision energies for MS/MS fragmentation were set to 20, 50, and 70 V. Full MS scans were acquired at a resolution of 60,000, whereas MS/MS scans were acquired at a resolution of 15,000.

Data processing, metabolite annotation, and statistical analysis

Peak deconvolution and alignment were performed using MS-DIAL (version 5.5), and feature tables were exported in Excel format for further analysis. For metabolite annotation, libraries were searched against public MS/MS spectral libraries including MS-DIAL's internal library, GNPS, and MoNA (MassBank of North America). Annotation was performed in accordance with the Metabolomics Standards Initiative (MSI), with all identified compounds classified at Level 2 confidence, except for glutathione, which was designated as Level 3 due to its identification being based exclusively on MS1 accurate mass and isotopic pattern in the absence of confirmatory MS2 spectral data (Supplementary Table S1, Supplementary Table S2). Peak areas were normalized to the ISTD. Data preprocessing included total ion current normalization, log-transformation, and Pareto scaling, conducted through MetaboAnalyst 6.0 (https://www.metaboanalyst.ca). Raw LC–MS chromatograms and MS spectra were reviewed using Xcalibur (Qual Browser, Thermo Fisher Scientific). Multivariate statistical analyses and metabolite pathway enrichment analyses were performed in MetaboAnalyst 6.0, while lipid-specific enrichment was conducted using the Lipid Pathway Enrichment Analysis platform (LIPEA, https://hyperlipea.org). Statistical significance was determined by Student's t-test, with $p < 0.05$ considered significant. Data visualization was performed using Microsoft Excel 2016 (Microsoft, Seattle, WA, USA) and Python 3.11 with Matplotlib and Seaborn libraries. To explore the functional relationships among significantly altered plasma metabolites, pairwise correlation analysis was performed using the Pearson correlation coefficient across all samples. A correlation threshold of $|r| \geq 0.6$ was applied to retain biologically relevant associations. From the resulting correlation matrix, the top 20 metabolite pairs with the highest absolute correlation values were selected for visualization. A chord diagram was constructed to depict these pairwise relationships using the Holoviews library (v1.18.3) in Python 3.11.

Results

Multivariate statistical analysis of breed and regional differences

A mass spectrometry-based untargeted metabolomics platform was employed, yielding a total of 41,688 metabolic features. To investigate breed and regional metabolic differences, multivariate statistical analysis was performed using plasma metabolomic profiles obtained from 49 Thoroughbred horses from Seoul Racecourse and 50 Jeju Native Horses from Jeju Racecourse. Principal component analysis (PCA) of the plasma metabolomic profiles revealed clear separation between the two groups at the 95% confidence level (Fig. 2A). In contrast, a PCA model stratified by sex (female, male, and gelding) demonstrated completely overlapping clusters with substantial overlap among the sex groups, suggesting that sex did not produce a clear separation in the global metabolic profiles (Supplementary Fig. S1). To further characterize the separation between the two populations, partial least squares-discriminant analysis (PLS-DA) was performed (Fig. 2B). To evaluate the reliability of the PLS-DA model, principal component 1 and principal component 2 were found to explain 27.7% and 11.3% of the total variance, respectively. Cross-validation yielded a classification accuracy of 1.00, indicating excellent discriminative power. The coefficient of determination (R^2) was 0.99076, and the predictive ability (Q^2) was 0.98802, indicating high model fit and predictive performance. To assess the significance of the classification and evaluate the risk of overfitting, a permutation test with 100 iterations was performed. None of the permuted models outperformed the original model, and the empirical p-value was found to be less than 0.01. Peak features were filtered using an absolute $\log_2$ fold change ≥ 2.0 and a p-value < 0.05. Using these criteria, a total of 11,545 differential features were retained, of which 4,810 were higher in abundance in the Seoul Thoroughbred group, while 6,735 were higher in abundance in the Jeju native horse group (Fig. 3A). Metabolites that contributed significantly to group differences were further filtered using a threshold VIP (Variable Importance in Projection) score of ≥ 1.0 derived from the PLS-DA model. As depicted in the volcano plot (Fig. 3A) and detailed in Table 1, 18 highly significant circulating metabolites were isolated. The distinct baseline expression patterns of these key differential metabolites —ranging from lower-abundance triglycerides to higher-abundance *N*-acetyl-L-aspartate in the Seoul group—are further summarized by their mean normalized intensities in a centroid heatmap (Supplementary Fig. S1). Among these, five metabolites were markedly higher in abundance in the Seoul group, notably *N*-acetyl-L-aspartate ($\log_2$(FC) = 8.9) and adenosine ($\log_2$(FC) = 7.0). Conversely, 13 metabolites showed significantly lower relative abundance in the Seoul cohort. This lower circulating metabolite pool was characterized by substantially lower levels of polyamines (spermine, $\log_2$(FC) = -13.1), various triglycerides (e.g., TG (14:1_16:0_16:1), $\log_2$(FC) = -12.2), as well as tentatively identified glutathione ($\log_2$(FC) = -4.7), glutamate ($\log_2$(FC) = -2.6), and citric acid ($\log_2$(FC) = -2.2).

Table 1
Differentially abundant metabolites between Seoul and Jeju horses

No.	Identification	VIP	Log_2(FC)	p-value
4225	*N*-Acetyl-L-aspartate	1.7	8.9	**
29220	DG(37:7)	2.2	8.5	**
8204	Adenosine	3	7	**
7131	Ubiquinone-1	1.7	4.8	**
8596	NVE_274.1802_16.6	1.6	3.8	**
963	Taurine	1.4	-2.1	**
34578	PC(18:1_18:2)	1.1	-2.2	**
5064	Citric acid	1.5	-2.2	**
1782	Glutamate	1.6	-2.6	**
26494	Bilirubin	1.4	-2.6	**
34371	PC(O-37:5)	2	-4	**
10907	Glutathione [a]	1.6	-4.7	**
33870	TG(14:0_14:0_16:1)	2.3	-6.2	**
34855	TG(14:0_16:1_16:1)	2.6	-7.2	**
33869	TG(14:0_14:1_16:0)	2.6	-8.5	**
34386	TG(14:0_15:0_16:1)	3.4	-10.1	**
34857	TG(14:1_16:0_16:1)	3.6	-12.2	**
4485	Spermine	4.1	-13.1	**

Note: Metabolites were selected based on VIP ≥ 1, $|log_2(FC)| \geq 2$, and $P < 0.05$. *$P < 0.05$, **$P < 0.01$. Abbreviations: VIP, variable importance in projection; FC, fold change; DG, diacylglycerol; PC, phosphatidylcholine; TG, triglycerides. [a] Tentatively annotated based on accurate MS1 mass and isotopic pattern (MSI Level 3).

Pathway enrichment analysis of differential metabolites

To contextualize these molecular perturbations biologically, pathway enrichment analysis was performed against the Equus caballus KEGG database (Fig. 3B). This analysis identified differences in pathways related to central carbon and nitrogen metabolism between the two groups. The most significantly enriched pathways (-log(p) > 1.0) were related to amino acid metabolism, energy metabolism, and redox-related processes. Specifically, the enriched pathways included alanine,

aspartate and glutamate metabolism, arginine biosynthesis, the TCA cycle, and folate-mediated one-carbon metabolism. Furthermore, distinct secondary enrichments were observed in porphyrin metabolism and taurine/hypotaurine metabolism, directly reflecting the significant baseline alterations in circulating bilirubin and taurine levels.

While several lipid-centric networks, such as alpha-linolenic acid, linoleic acid, glycerophospholipid, and arachidonic acid metabolism, alongside retrograde endocannabinoid signaling and choline metabolism in cancer, also yielded statistical enrichment (Supplementary Table S3), these had to be interpreted with caution. The statistical significance for these lipid modules was driven by a highly limited mapping ratio, often featuring only a single tentatively identified compound out of a total of 5 to 75 constituent pathway members. Consequently, these broad lipid-related pathways were presented for reference purposes only and were excluded from the primary mechanistic interpretation.

Metabolic correlation and clustering analysis

To investigate coordinated metabolic relationships across the study population, a pairwise Pearson correlation analysis was performed on plasma metabolites. The top 20 pairs showing the strongest correlations ($|r| \geq 0.6$) were visualized (Fig. 4A). Notably, amino acids such as proline, glutamate, methionine, and L-tryptophan exhibited strong positive correlations and formed a tightly connected module, suggesting involvement in nitrogen and amino acid metabolism. Similarly, intermediates of the TCA cycle, including malate and citrate, also showed close correlations. Hierarchical clustering heatmap analysis of significantly altered metabolites further confirmed a clear separation between the Seoul Thoroughbred and Jeju native horse groups based on their metabolite expression profiles (Fig. 4B).

Discussion

In this study, high-resolution untargeted metabolomics successfully revealed distinct baseline plasma metabolic profiles between Seoul Thoroughbreds and Jeju Native Horses, driven by their contrasting genetics and localized rearing environments. Our findings demonstrate that dietary composition and management practices are strongly associated with variations in systemic metabolic networks of equine athletes [13, 18]. As illustrated in Fig. 5, pathway enrichment highlighted marked alterations in key metabolic axes, notably nitrogen/amino acid metabolism, TCA cycle-mediated energy homeostasis, and the methionine cycle.

Significant variations in plasma amino acid profiles between Seoul Thoroughbreds and Jeju Native Horses are closely linked to the interplay between dietary composition and athletic metabolic demands [19, 20]. The concentrate-based, high-starch feeding regimen of the Seoul group and its associated hindgut microbial shifts [6] alter systemic amino acid availability, whereas the higher exercise intensity of elite Thoroughbreds heavily engages the arginine-citrulline-glutamate/glutamine axis to manage nitrogen excretion and oxidative stress regulation, which is consistent with the observed alterations in tentatively annotated glutathione [21, 22].

In addition to nitrogen handling, systemic energy metabolism may represent another major axis of metabolic difference between the two populations. Thoroughbreds exhibit a relatively high proportion of fast-twitch fibers with enhanced glycolytic and anaerobic capacity, whereas Jeju Native Horses possess a higher proportion of oxidative Type I fibers, reflecting distinct muscular metabolic phenotypes [11, 23]. Furthermore, differences in muscle characteristics may coexist with differences in dietary substrate availability, including glucose and starch-derived substrates in concentrate-fed Thoroughbreds and volatile fatty acids generated through forage fermentation in the hindgut [6, 24]. These differences in muscle phenotype and substrate availability may be associated with alterations in systemic energy metabolism and differences in circulating TCA cycle-related metabolites such as citrate and malate.

Furthermore, the alterations observed in the folate-dependent one-carbon metabolic pool point to differences in pathways related to nutritional inputs, methyl-group metabolism, and cellular redox homeostasis between the two equine populations [25, 26]. The folate-dependent one-carbon cycle is intrinsically coupled with the methionine cycle, which regulates S-adenosylmethionine (SAM) availability and provides a biochemical link between dietary inputs and methyl-group metabolism. Differences in circulating methionine may be associated with changes in methyl-group availability and may have implications for methylation-related processes [5, 27]. Given the differences in chronological age, training history, and management conditions between the groups, the observed metabolic differences may also be related to age- and exercise-associated metabolic variation reported in horses [28–30]. Moreover, because the one-carbon network intersects with serine–glycine interconversion and the transsulfuration pathway, alterations in these pathways may be associated with differences in amino acid metabolism and cellular antioxidant capacity. Although glutathione was tentatively annotated in this study, the observed pathway patterns were consistent with differences in metabolic processes related to redox homeostasis [26, 31].

Despite these compelling metabolic insights, several limitations of this study should be acknowledged. First, the retrospective use of residual clinical specimens collected under field racecourse conditions meant that key variables, including breed (Thoroughbred vs. Jeju Native Horse), chronological age (mean 4.3 vs. 6.1 years), and dietary regimens (concentrate-based vs. forage-oriented), were not independently controlled or perfectly matched between the two groups. These factors represent composite characteristics of real-world racecourse management; therefore, the findings should be interpreted as field-based metabolic associations rather than as evidence from a strictly controlled experimental design. Although the two populations showed consistent separation in multivariate analyses, the present study cannot determine the independent contributions of breed, environment, age, diet, sex, or other management-related factors to the observed metabolic differences. Future studies using controlled designs and better-matched groups will therefore be needed to investigate the independent contributions of breed, age, diet, exercise, and environmental conditions to equine metabolic variation. Second, while untargeted metabolomics enabled comprehensive mapping of global baseline plasma metabolic alterations, it fundamentally provides a top-down systemic observation and does not directly establish the underlying causal mechanisms at the gastrointestinal level. In highly sensitive hindgut fermenters like equines, the shift between forage- and concentrate-based diets can be associated with

changes in the hindgut microbiome structure, subsequently modifying the production of volatile fatty acids (VFAs) and microbial amino acid metabolism. Therefore, future multi-omics studies—particularly integrating fecal metagenomics and metatranscriptomics to characterize hindgut microbial functional activity—alongside targeted VFA analysis, will be necessary to establish more definitive mechanistic links between dietary interventions, hindgut microbial fermentation, and the resulting systemic metabolic phenotypes.

Conclusion

This study provides a comprehensive and high-resolution metabolomic mapping of baseline plasma profiles in Thoroughbreds and Jeju Native Horses, revealing distinct systemic metabolic phenotypes associated with breed-specific characteristics and contrasting rearing environments. Using an untargeted metabolomics workflow and multi-pathway enrichment analysis, we identified differences involving nitrogen and amino acid metabolism, TCA cycle-associated energy metabolism, and folate-dependent one-carbon and redox-related pathways. These findings are consistent with differences in nutritional management and other population-level characteristics, including muscular and environmental factors, that may be associated with systemic metabolic profiles in horses. Ultimately, this work establishes a valuable baseline framework for equine metabolomics and provides broader insights into metabolic plasticity in mammalian athletes under varying nutritional, environmental, and physiological conditions.

Abbreviations

ACN
Acetonitrile
DDA
Data-Dependent Acquisition
DG
Diacylglycerol
ESI
Electrospray Ionization
FA
Formic Acid
FC
Fold Change
GNPS
Global Natural Products Social Molecular Networking
ISTD
Internal Standard
KEGG
Kyoto Encyclopedia of Genes and Genomes

KRA
Korea Racing Authority
LC–MS
Liquid Chromatography–Mass Spectrometry
LIPEA
Lipid Peak Assignment
MoNA
MassBank of North America
MS
Mass Spectrometry
MS/MS
Tandem Mass Spectrometry
MS-DIAL
Mass Spectrometry-Data Independent Analysis
MSI
Metabolomics Standards Initiative
NMR
Nuclear Magnetic Resonance
PCA
Principal Component Analysis
PC
Phosphatidylcholine
PLS-DA
Partial Least Squares-Discriminant Analysis
SAM
S-Adenosylmethionine
TG
Triglycerides
TCA
Tricarboxylic Acid
UHPLC
Ultra-High-Performance Liquid Chromatography
VIP
Variable Importance in Projection

Declarations

Ethics approval and consent to participate

The Korea Racing Authority Institutional Animal Care and Use Committee (IACUC) determined that this study was exempt from IACUC review and approval because it used only residual plasma samples originally collected during routine equine doping control activities. No additional animals were used, no additional biological specimens were collected, and no animal procedures or interventions were performed for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

YB Kwak carried out the conceptualization, investigation, data analysis, writing – original draft, and writing – review & editing. The author approved the final manuscript and agreed to be accountable for all aspects of the work.

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Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

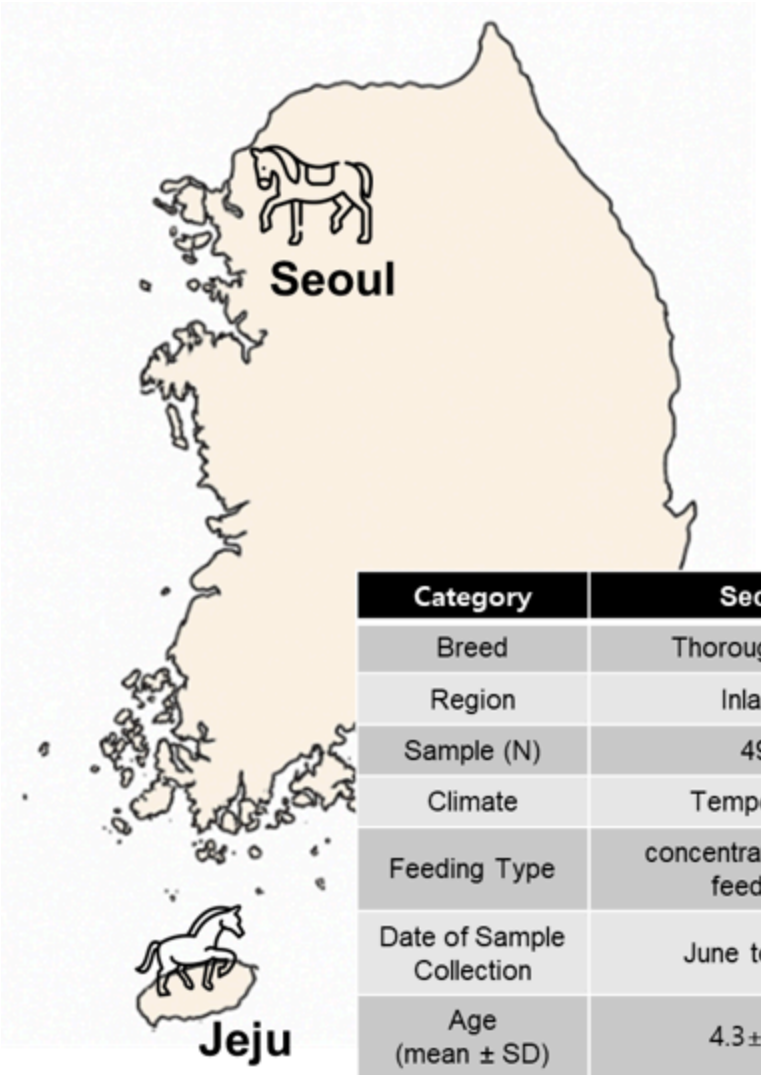
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Figures



Category	Seoul	Jeju
Breed	Thoroughbred	Jeju Native Horse
Region	Inland	Island
Sample (N)	49	50
Climate	Temperate	Maritime
Feeding Type	concentrate-based feeding	forage-based diet with free access to pasture
Date of Sample Collection	June to July	June to July
Age (mean ± SD)	4.3±1.2	6.1±2.4
Sex distribution (M:F:G)	18:23:8	10:12:28

Figure 1

Geographical and phenotypic overview of the horse populations used in this study.

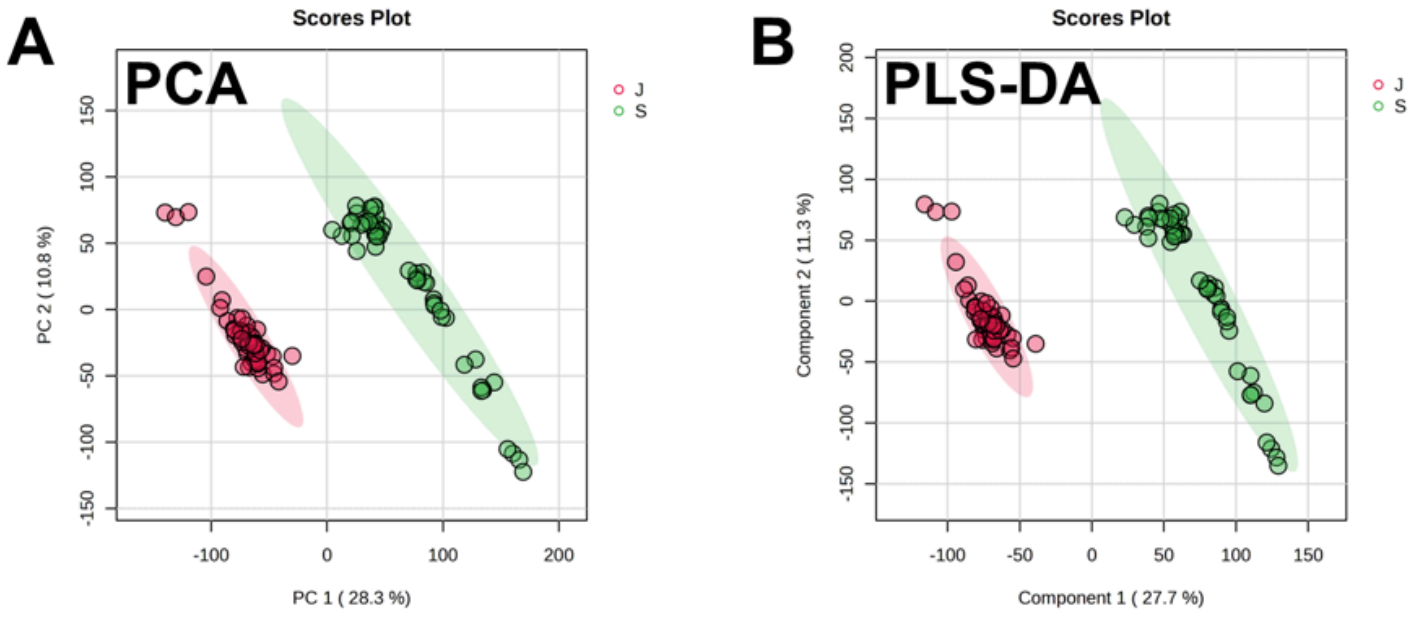


Figure 2

Scores plots of (A) principal component analysis (PCA) and (B) partial least squares-discriminant analysis (PLS-DA) based on untargeted metabolomic profiles of plasma from Seoul (S, n = 49) and Jeju (J, n = 50) horses. Both models reveal clear separation between the two groups, indicating distinct metabolic phenotypes. Ellipses represent 95% confidence intervals.

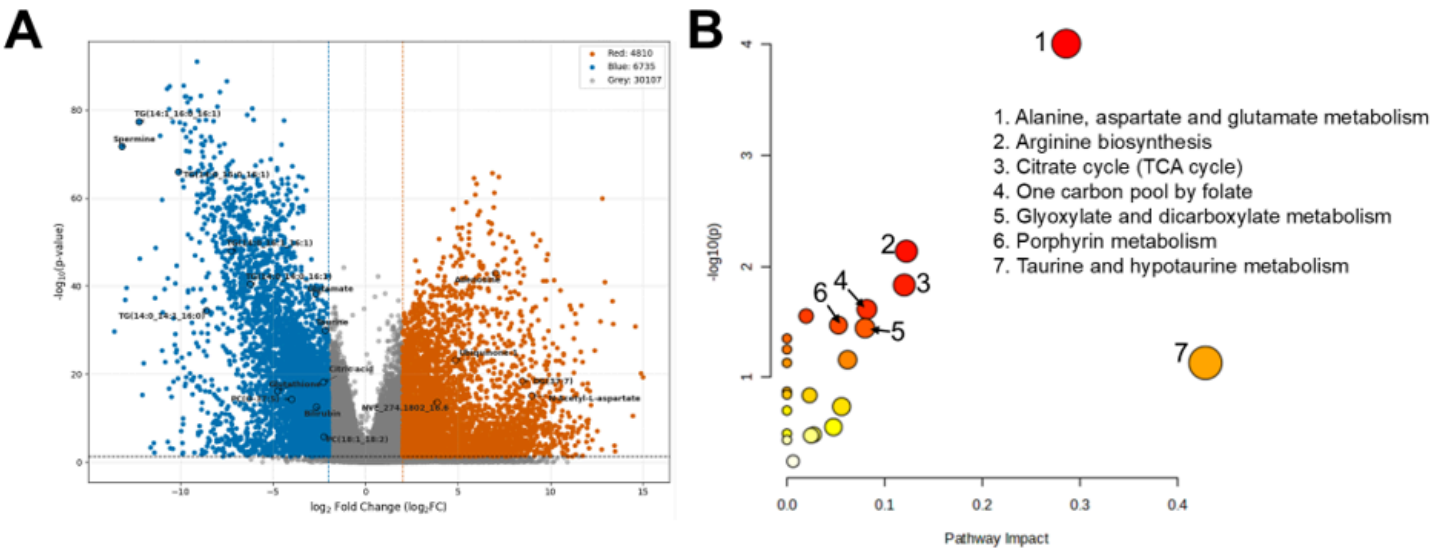


Figure 3

(A) Volcano plot showing differentially expressed plasma metabolites between Seoul and Jeju horses based on $\log_2$ fold change and p-value thresholds. (B) KEGG pathway enrichment analysis of *Equus*

caballus using significantly altered metabolites. Pathways with −logP ≥ 1 and p < 0.05 were considered significant based on over-representation analysis. Pathway annotations were based on the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa Laboratories).

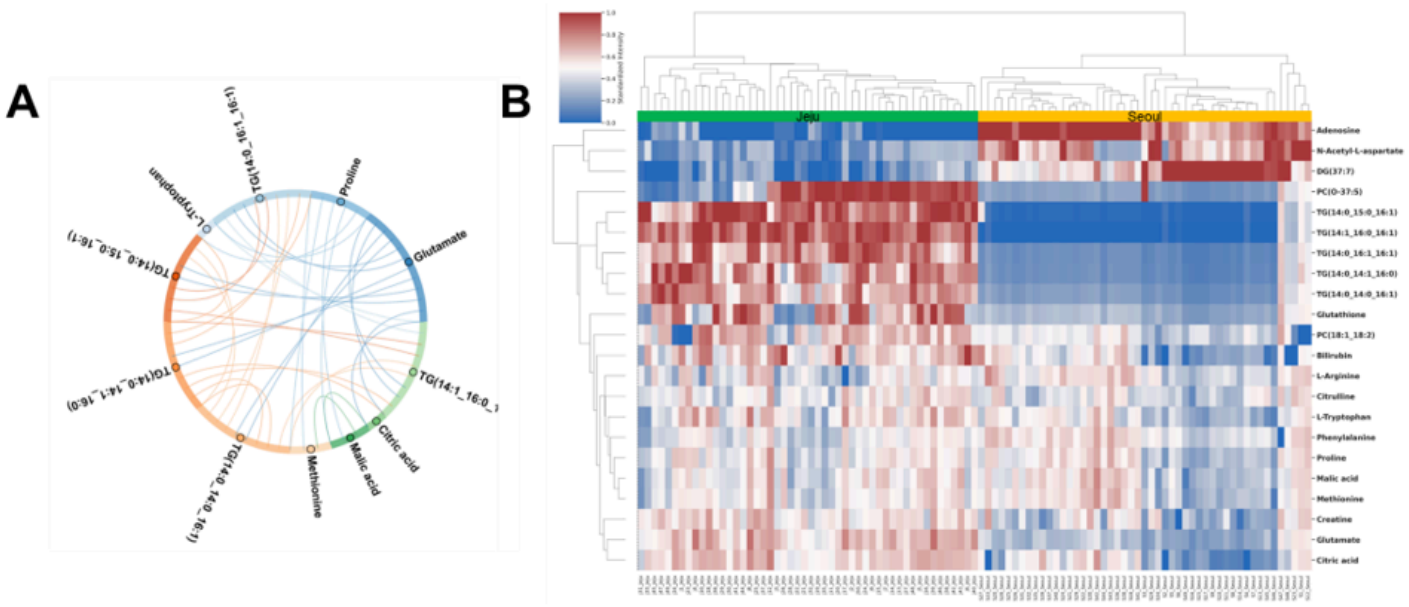


Figure 4

(A) Chord diagram depicting top correlated metabolite interactions (|r| ≥ 0.6). (B) Hierarchical clustering heatmap illustrating the relative expression patterns and distinct sample-wise segregation of key differential metabolites between Seoul Thoroughbreds and Jeju Native Horses.

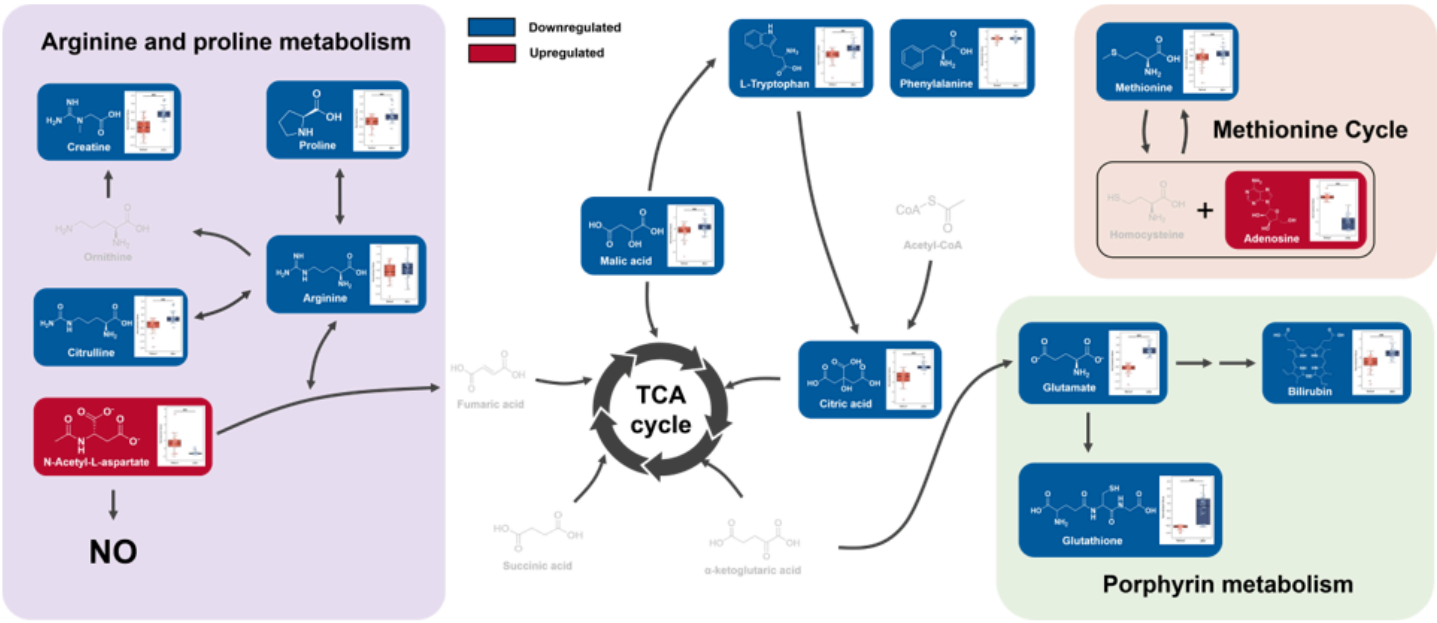


Figure 5

Schematic organization of metabolic pathways for plasma metabolites between Seoul and Jeju horses.

Supplementary Files

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- SupplementaryTables.docx