

Dietary net energy regulates puberty onset and estrus synchrony of replacement gilts via remodeling gut microbiota and serum metabolic profiling

Junwen Wang

Huazhong Agricultural University

Jiajian Tan

Guangxi YangXiang Joint Stock Company

Haiqing Sun

Guangxi YangXiang Joint Stock Company

Haidi Jiang

Guangxi YangXiang Joint Stock Company

Hongkui Wei

Huazhong Agricultural University

Jian Peng

Huazhong Agricultural University

Yuanfei Zhou

`zhouyuanfei@mail.hzau.edu.cn`

Huazhong Agricultural University

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Abstract

Background Optimal net energy (NE) provision during the rearing period is critical for regulating reproductive development of replacement gilts, while the mechanistic links between dietary NE, gut microbiota and host metabolism remain to be clarified. Herein, we investigated phase-specific NE requirements and explored the regulatory mechanisms using untargeted metabolomics and gut metagenomics. In total, 366 Danish Landrace × Large White crossbred gilts were allocated to five graded NE treatments. The NE levels of T1–T5 were 2,090, 2,180, 2,270, 2,360 and 2,450 kcal/kg (120–156 d), and revised to 2,040, 2,130, 2,220, 2,310 and 2,400 kcal/kg from 157 d to pre-breeding.

Results The results showed that gilts fed the moderate NE diet (T4: 2,360 kcal/kg in Phase 1 and 2,310 kcal/kg in Phase 2) exhibited the highest average daily gain and feed conversion efficiency. T4 and T5 attained puberty 6–8 d earlier than low-NE groups, and T4 showed the highest estrus rate (81.94%) before 200 d. Its backfat thickness at first mating fell within the optimal reproductive range, along with increased dynamic secretion of luteinizing hormone and follicle-stimulating hormone. Dietary NE did not affect litter size, farrowing rate or retention rate across the first two parities. Metabolomic analysis identified 107 differential metabolites were identified between T2 and T4, mainly related to glycerophospholipid, triglyceride, organic acid and small peptide metabolism. The two groups presented divergent gut microbial structures with unchanged alpha diversity. T4 was enriched with *Streptococcus gallolyticus* and *Streptococcus equinus*, and had enhanced pathways of carbohydrate catabolism, ABC transporter, amino acid and vitamin biosynthesis. The enriched microbiota regulated energy and lipid metabolism to activate the hypothalamic–pituitary–ovarian axis and synchronize puberty.

Conclusions Collectively, phase-adjusted NE improves growth and puberty synchrony via remodeling the gut microbiota-metabolism axis, with no adverse effects on early-parity reproduction. This work offers a practical nutritional strategy and multi-omics basis for modern replacement gilt management.

Introduction

Sow longevity and lifetime productivity are pivotal determinants of economic efficiency in modern intensive swine production. Excessive involuntary culling of replacement gilts substantially impairs overall herd productivity, with a considerable share of gilt removals stemming from delayed puberty, persistent anestrus, and unsynchronized sexual maturation [1]. Our prior investigations, alongside reports from independent research groups, have documented that involuntary culling rates ranging from 52.8% to 62.76% in low-parity females represent a primary constraint limiting sow lifetime productivity, with anestrus failure being a predominant culling reason for replacement gilts [2–3]. Reasonable gilt replacement ensures genetic progress, whereas sufficient retention rate is required to exert full reproductive potential across successive parities.

Nutritional strategies implemented throughout the replacement gilt rearing phase exert profound regulatory effects on somatic growth, body composition, skeletal integrity, and reproductive tract development [4]. Age, body weight (BW) and backfat thickness (BF) at first estrus are tightly correlated with subsequent two-parity reproductive performance and herd retention rate [5–6]. Our previous work further demonstrated that gilts exhibiting first estrus before 210 d of age, with body weight of 115–124.9 kg and backfat thickness of 14–15 mm, exhibit superior reproductive traits [7]. Despite these established target phenotypic benchmarks, systematic dose-response trials defining optimal dietary nutrient specifications to support targeted puberty timing and ideal first-mating body status are currently lacking.

Dietary energy functions as a central nutritional modulator governing nutrient partitioning and metabolic resource allocation during gilt developmental physiology [8–9]. Dynamic energy homeostasis imbalance triggers hierarchical resource reallocation, prioritizing somatic maintenance over reproductive programming [10]. Distinct dietary energy supply regimens directly modulate activation of the hypothalamic–pituitary–ovarian (HPO) axis; suppressed HPO signaling delays estrus initiation or induces anestrus, ultimately elevating involuntary culling risks for replacement gilts [11]. Over the past three decades, intensive genetic selection has reshaped key reproductive traits of modern sow lines, advancing puberty onset by 30 to 45 days while improving ovulation capacity and total litter size [12]. Although nutritional standards from NRC (2012) [13], DanBred (2023) [14] and Genesus (2023) [15] have been proposed, obvious discrepancies exist among current recommendations. The metabolic phenotype of genetically improved modern gilts has fundamentally changed, whereas conventional feeding protocols fail to match genetic progress, forming a critical bottleneck restricting production efficiency.

Most existing studies have focused on phenotypic responses of gilt growth and reproduction to dietary energy, while the intrinsic mechanism underlying nutritional regulation remains unclear from the perspective of gut microbiota and host metabolic profiling. Gut microbiota acts as a master regulator of nutrient digestibility, energy extraction efficiency, and bioactive metabolite biosynthesis [16], while serum metabolome profiling captures systemic whole-body physiological and metabolic signatures [17]. Integrated multi-omics analysis provides a powerful approach to uncover the molecular cascade linking nutritional intervention to reproductive development [18].

Accordingly, the present experiment aimed to evaluate the effects of graded dietary NE levels during rearing on growth performance, puberty characteristics, circulating reproductive hormone concentrations, estrus synchronization uniformity, and reproductive performance across the first two parities in Danish Landrace × Large White replacement gilts. Low-NE and optimal-NE groups were further selected for untargeted metabolomics and gut metagenomics to profile differential metabolites, microbial composition and functional pathways, followed by microbe–metabolite correlation analysis. We hypothesized that moderate dietary NE could accelerate puberty onset and improve estrus synchronization via remodeling gut microbial structure and host metabolic homeostasis, without impairing reproductive performance in the first two parities. Collectively, these findings seek to establish a precision nutritional feeding framework tailored to modern replacement gilt rearing, and furnish multi-omics mechanistic evidence underpinning nutritional regulation of gilt reproductive development.

Materials and methods

Animals and experimental design

All animal procedures were approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University (approval number: HZAUSW-2023-0027) and conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

A total of 366 Danish Landrace × Large White crossbred replacement gilts with initial age of 127.5 ± 4.8 d and initial BW of 66.3 ± 6.4 kg were used in this trial. Gilts were individually raised in gestation stalls (2.0×0.6 m) equipped with a wet-dry feeder under ad libitum feeding. The ambient temperature was maintained at $22 \pm 2^\circ\text{C}$ with a 16-h light regime. Estrus detection was performed twice daily at 08:00 and 16:00 from 160 d of age via direct boar contact for 15 min. Standing reflex upon boar mounting was defined as the first estrus.

The rearing trial was divided into two phases: phase 1 from 120 to 156 d of age, and phase 2 from 157 d to pre-breeding. Gilts were allocated to five treatments using stratified randomization based on initial BW, with 72–75 gilts per group. Individual gilt was regarded as the experimental unit for growth, puberty and reproductive parameters. Feed intake was individually recorded using the FIRE electronic feeding system to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed-to-gain ratio (F:G). The number of diseased and dead gilts was recorded throughout the trial. Sample size changes for each indicator are presented in Table 1. Estrus rates before 200 and 220 d of age and gilt selection rate were statistically calculated.

Table 1
Changes in the experimental sample sizes across different indicators

Item	T1	T2	T3	T4	T5	Total
No. of selected replacement gilts, Head	73	72	75	72	74	366
Anestrous, Head	5	9	3	4	5	26
Spontaneous disease, Head	0	0	3	1	0	4
Missing Records, Head	2	0	4	5	2	13
No. of gilts at first mating, Head	66	63	65	62	67	323

Table 2
Composition and nutrient levels of experiment diets

Item	Phase 1 (120–156 d)					Phase 2 (from 157 d to breeding)				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Ingredients (as-fed basis, %)										
Corn		6.20	8.80	30.10	57.03		3.40	9.60	14.40	39.42
Barley	44.59	44.92	49.97	31.19	2.50	50.10	50.03	50.05	50.04	23.70
Sorghum	20.00	20.00	20.00	20.00	20.00	16.90	20.00	20.00	20.00	20.00
Wheat bran	22.60	14.00	4.40			22.70	14.70	6.70		
Soybean meal	9.90	11.90	13.80	15.60	16.80	7.50	9.00	10.70	12.20	13.30
Soybean oil					0.50				0.34	0.50
Salt	0.43	0.44	0.45	0.46	0.46	1.50	1.45	1.39	1.35	1.34
Limestone	1.48	1.43	1.37	1.33	1.32	0.28	0.43	0.58	0.71	0.78
Dicalcium phosphate	0.27	0.43	0.60	0.74	0.82	0.43	0.44	0.45	0.46	0.46
Choline chloride (50%)	0.10	0.10	0.10	0.10	0.10	0.08	0.08	0.08	0.08	0.08
L-Lysine (78%)	0.24	0.21	0.17	0.16	0.15	0.17	0.15	0.13	0.10	0.10
DL-Methionine(99%)	0.02	0.02	0.01			0.01				
L-Threonine (98.5%)	0.05	0.03	0.01			0.01				
Mineral and vitamin premix ¹	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Total	100	100	100	100	100	100	100	100	100	100
Nutrient content (as-fed basis, %)										
NE, Kcal/kg ²	2090	2180	2270	2360	2450	2040	2130	2220	2310	2400
Crude protein ³	13.98	14.11	13.96	14.04	13.96	13.16	13.12	13.12	12.96	13.02
Standardized ileal digestible amino										
¹ Premix supplied per kg diet: vitamin A, 8400 IU; vitamin D ₃ , 1680 IU; vitamin E, 70 mg; vitamin K ₃ , 3.4 mg; vitamin B ₁ , 1.85 mg; vitamin B ₂ , 6 mg; vitamin B ₆ , 3 mg; vitamin B ₁₂ , 30 µg; D-pantothenic acid, 21 mg; nicotinic acid, 32 mg; D-biotin, 0.44 mg; folic acid, 1.7 mg; Zn (ZnSO ₄ ·H ₂ O), 60 mg; Fe (FeSO ₄ ·H ₂ O), 140 mg; Mn (MnSO ₄ ·H ₂ O), 50 mg; Cu (Cu ₂ (OH) ₃ Cl), 17.5 mg; I (Ca(IO ₃) ₂), 1.5 mg; Se (Na ₂ SeO ₃), 0.34 mg.										
² Net energy and standardized ileal digestible amino acid were calculated was calculated using the BRILL software, based on the 2012 version of the INRA-AFZ Feedipedia database (standard French feed ingredient database).										
³ Values determined by analysis; each value is based on triplicate determinations.										

Item	Phase 1 (120–156 d)					Phase 2 (from 157 d to breeding)				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
acid ²										
Lysine	0.65	0.65	0.65	0.65	0.65	0.55	0.55	0.55	0.55	0.55
Methionine	0.19	0.20	0.19	0.19	0.20	0.17	0.16	0.17	0.17	0.18
Methionine+Cysteine	0.20	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19	0.19
Threonine	0.41	0.41	0.41	0.42	0.43	0.34	0.35	0.37	0.38	0.39
Tryptophan	0.15	0.15	0.15	0.14	0.13	0.14	0.14	0.14	0.13	0.13
Valine	0.51	0.53	0.55	0.56	0.56	0.47	0.49	0.50	0.52	0.52
Isoleucine	0.42	0.44	0.46	0.48	0.49	0.38	0.40	0.42	0.43	0.44
Calcium ³	0.60	0.64	0.66	0.64	0.64	0.66	0.67	0.67	0.68	0.69
Total phosphorus ³	0.52	0.50	0.47	0.46	0.46	0.55	0.53	0.50	0.47	0.49
¹ Premix supplied per kg diet: vitamin A, 8400 IU; vitamin D ₃ , 1680 IU; vitamin E, 70 mg; vitamin K ₃ , 3.4 mg; vitamin B ₁ , 1.85 mg; vitamin B ₂ , 6 mg; vitamin B ₆ , 3 mg; vitamin B ₁₂ , 30 µg; D-pantothenic acid, 21 mg; nicotinic acid, 32 mg; D-biotin, 0.44 mg; folic acid, 1.7 mg; Zn (ZnSO ₄ ·H ₂ O), 60 mg; Fe (FeSO ₄ ·H ₂ O), 140 mg; Mn (MnSO ₄ ·H ₂ O), 50 mg; Cu (Cu ₂ (OH) ₃ Cl), 17.5 mg; I (Ca(IO ₃) ₂), 1.5 mg; Se (Na ₂ SeO ₃), 0.34 mg.										
² Net energy and standardized ileal digestible amino acid were calculated using the BRILL software, based on the 2012 version of the INRA-AFZ Feedipedia database (standard French feed ingredient database).										
³ Values determined by analysis; each value is based on triplicate determinations.										

Diet and feeding management

- The experimental diets covered the recommended NE ranges for replacement gilts according to NRC (2012) [13], DanBred (2023) [14] and Genesus (2023) [15]. Five NE gradient treatments were designed across two rearing phases. Diet ingredient composition is shown in Supplementary Table 2. In phase 1 (120–156 d), dietary NE levels were 2,090, 2,180, 2,270, 2,360 and 2,450 kcal/kg, with crude protein (CP) of 14.0% and standardized ileal digestible (SID) lysine of 0.65% in all treatments. In phase 2 (157 d to pre-breeding), NE levels were adjusted to 2,040, 2,130, 2,220, 2,310 and 2,400 kcal/kg, while CP and SID lysine were reduced to 13.0% and 0.55%, respectively.

Blood sample collection

To evaluate the effect of dietary NE on ovarian follicle development, 10 gilts with average BW were selected from each group on day 17 after the first estrous cycle [19]. Between 08:00 and 14:00, 8 blood samples were collected over 6 hours: Within 30 minutes before feeding, followed by intervals of 30, 60, 105, 150, 210, 270, and 330 minutes post-prandially. Each 5 mL blood sample was drawn into heparinized vacuum tubes, immediately placed on ice, and

centrifuged at $2,400 \times g$ for 15 minutes with 4°C . The supernatant was aliquoted into 1 mL Eppendorf tubes labeled with the sow's ear tag number and sampling time, then temporarily stored at -20°C and long-term stored at -80°C .

Reproductive data

Litter farrowing and weaning performance of each sow were recorded for the first two parities. Total number born (TNB), number born alive (NBA) and number weaned (NW) were calculated. The number of mated sows, farrowed sows and re-mated sows were recorded to determine farrowing rate and retention rate for each parity.

Hormones analysis

Measurement of peak levels and pulses of luteinizing hormone (LH) (ml296525) and follicle-stimulating hormone (FSH) (ml002326), as well as levels of estradiol (E2) (ml002366), progesterone (ml002422), insulin (ml002341), insulin-like growth factor-1 (IGF-1) (ml002344), and leptin (ml002355) in blood samples using non-competitive enzyme-labeled immunosorbent assays (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China). All hormone assays were performed in 96-well plates using an enzyme-labeled meter (TECAN Infinite M200 PRO, Männedorf, Switzerland), according to the manufacturer's protocol.

Non-targeted Metabolomics Analysis

Non-targeted metabolomics was performed on T2 and T4 groups using liquid chromatography-mass spectrometry (LC-MS). Samples were extracted with organic solvents, centrifuged, reconstituted, and detected. Raw data were preprocessed by peak picking, alignment, denoising, and normalization. Principal component analysis (PCA) was used to evaluate global metabolic separation. Variable importance in projection (VIP) was calculated via orthogonal partial least squares discriminant analysis (OPLS-DA). Significantly differential metabolites were identified with thresholds of $\text{VIP} > 1$, $|\log_2\text{FC}| > 1$, and $P < 0.05$. Differential metabolites were standardized by Z-score and subjected to hierarchical clustering, and annotated by chemical classifications including lipids, organic acids, phenolics, heterocyclic compounds, small peptides, and nucleotide derivatives.

Metagenomics Analysis

Microbial community structure, diversity and functional pathways were analyzed by high-throughput metagenomic sequencing in T2 and T4 groups. Genomic DNA was extracted, quantified, and quality-checked, followed by sequencing library construction and paired-end sequencing on the Illumina platform. Raw reads were filtered to remove low-quality and host-derived sequences. Clean reads were assembled, and genes were predicted to construct a non-redundant gene catalog. Taxonomic annotation was performed at phylum, class, order, family, genus and species levels using reference databases.

Alpha diversity was estimated using Chao1 (richness) and Shannon (diversity) indices, with statistical comparisons between groups. Beta diversity was analyzed by principal coordinate analysis (PCoA) based on Bray–Curtis distance to visualize community separation. LEfSe and Metastats were used to identify differential taxa with thresholds of LDA Score > 3 and $P < 0.05$. Functional annotation and pathway enrichment were performed against the KEGG database, and differential pathways were identified at $P < 0.05$.

Statistical analysis

Calculation and statistical analyses were conducted using SAS software (SAS 9.4, SAS Institute Inc., Cary, NC, USA). Prior to evaluating differences between groups, the normality of the data was assessed using the Shapiro-Wilk test. The null hypothesis was rejected when $P < 0.05$, concluding that the population did not follow a normal distribution. For non-normally distributed reproductive hormone data, Box-Cox transformation was applied, including logarithmic, square root, and reciprocal transformations to achieve normality.

To analyze treatment effects, Linear regression was used to establish a linear relationship model between treatment and growth performance ($Y = \beta + \beta_1 X + \epsilon$). Quadratic regression was applied by introducing a squared term to the independent variable ($Y = \beta + \beta_1 X + \beta_2 X^2 + \epsilon$) to describe nonlinear relationships (e.g., parabolic trends). The significance of the quadratic coefficient β_2 was tested ($P < 0.05$) to determine whether a curvilinear effect existed between variables. Model performance was evaluated using adjusted R-squared and residual diagnostics.

For normally distributed plasma reproductive hormone levels between two groups, t-tests were performed using software, while one-way ANOVA was applied for comparing multiple normally distributed groups. The chi-square test was used to determine the independence of categorical variables or goodness-of-fit by comparing observed and expected frequencies. Critical values were determined based on degrees of freedom and compared with critical value tables to analyze differences in estrus rates between groups.

- Spearman rank correlation analysis was performed between key differential microbial species (metagenomics) and core differential metabolites (metabolomics). Correlation coefficient r was used to evaluate the strength and direction of associations. Correlations with $|r| > 0.5$ and $P < 0.05$ were considered significant, and the microbe-metabolite interaction network was constructed accordingly. A probability of $P < 0.05$ was considered statistically significant. All results were presented as mean $\pm$ standard deviation.

Results

Growth performance

Initial age and BW did not differ among five groups ($P > 0.05$) (Table 3). No significant differences were observed in BW at 156 d and puberty among treatments ($P > 0.05$). From the start to 156 d of age, ADFI was unaffected by NE level ($P > 0.05$), whereas ADG showed significant linear increase and convex quadratic response ($P < 0.01$), and F:G presented linear decrease and concave quadratic response ($P < 0.01$). The ADG of T4 and T5 was significantly higher than T2 and T3 ($P < 0.01$), while F:G of T5 was lower than T2 and T3 ($P < 0.01$).

Table 3

Effects of Dietary Net Energy Levels on Growth Performance of Replacement Gilts During Different Growth Stages

Item	T1	T2	T3	T4	T5	P-value		
						ANOVA	Linear	Quadratic
No. of gilts	72	70	71	72	70			
Initial days of age, d	127.53 ± 4.82	127.76 ± 4.80	127.63 ± 5.28	127.44 ± 4.43	127.72 ± 4.81	0.995	0.970	0.961
Initial BW, kg	66.34 ± 6.42	66.19 ± 6.79	66.33 ± 6.44	66.83 ± 5.99	66.17 ± 6.33	0.973	0.879	0.945
156-day BW, kg	91.16 ± 7.49	90.77 ± 7.68	90.77 ± 7.66	92.32 ± 7.48	91.84 ± 7.44	0.659	0.235	0.457
Pubertal BW, kg	125.33 ± 14.34	126.62 ± 16.17	125.15 ± 14.68	123.95 ± 16.21	125.15 ± 15.30	0.898	0.600	0.871
From initial days of age to 156 days of age								
ADFI, g/d	2308.66 ± 114.86	2303.08 ± 107.96	2316.86 ± 138.91	2329.17 ± 123.23	2310.62 ± 123.10	0.751	0.399	0.598
ADG, g/d	886.25 ± 86.93 ^{ab}	877.78 ± 101.92 ^b	872.81 ± 90.02 ^b	910.42 ± 100.65 ^a	916.89 ± 91.79 ^a	0.013	0.003	0.002
F:G	2.62 ± 0.23 ^{ab}	2.65 ± 0.30 ^a	2.68 ± 0.28 ^a	2.59 ± 0.29 ^{ab}	2.54 ± 0.23 ^b	0.014	0.007	0.001
From 160 days of age to puberty stage								
ADFI, g/d	2846.57 ± 135.58	2807.80 ± 174.20	2829.99 ± 179.46	2831.77 ± 157.73	2802.00 ± 151.82	0.446	0.279	0.556
ADG, g/d	886.58 ± 93.39 ^{bc}	874.72 ± 115.30 ^c	927.84 ± 149.21 ^b	982.90 ± 133.14 ^a	922.04 ± 158.64 ^b	< 0.01	< 0.01	0.001
F:G	3.24 ± 0.35	3.27 ± 0.49	3.21 ± 1.36	2.93 ± 0.39	3.28 ± 1.93	0.272	0.517	0.544
From the initial stage to puberty stage								
ADFI, g/d	2613.79 ± 129.16	2595.93 ± 141.13	2603.71 ± 167.00	2591.10 ± 141.78	2575.25 ± 127.93	0.573	0.124	0.297
ADG, g/d	882.89 ± 65.48 ^{cd}	871.87 ± 81.48 ^d	904.98 ± 67.63 ^{bc}	943.55 ± 84.24 ^a	923.20 ± 88.43 ^{ab}	< 0.01	< 0.01	< 0.01
F:G	2.97 ± 0.23 ^{ab}	3.00 ± 0.28 ^a	2.89 ± 0.24 ^{bc}	2.76 ± 0.23 ^d	2.81 ± 0.31 ^{cd}	< 0.01	< 0.01	< 0.01
BW = body weight; ADFI = average daily feed intake; ADG = average daily gain; F:G = feed-to-gain ratio. The data in the table are presented as mean ± standard deviation; Different lowercase letters (a, b, c, d) as superscripts within the same row indicate significant differences among groups ($P < 0.05$).								

From 157 d to puberty, ADFI and F:G were similar among groups ($P > 0.05$), but ADG exhibited significant linear and quadratic effects ($P < 0.01$). T4 had the highest ADG while T2 showed the lowest value ($P < 0.01$). Over the entire

rearing period, ADG and F:G followed consistent trends. T4 maintained higher ADG ($P < 0.01$) and lower F:G than T1, T2 and T3 ($P < 0.01$).

Puberty parameters and first mating parameters

As shown in Table 4, gilts in T4 and T5 reached puberty at 183.62 d and 184.11 d, respectively, which were significantly earlier than T1 (191.14 d), T2 (191.03 d) and T3 (189.63 d; $P < 0.01$). No differences were found in BW and backfat thickness (BF) at puberty among groups ($P > 0.05$). According to farm management, first mating was arranged after 220 d of age; thus NE level did not affect age and BW at first mating ($P > 0.05$) (Table 5). However, BF at first mating was significantly altered ($P < 0.01$). T4 had greater BF (14.52 mm) than T1 (13.86 mm) and T2 (13.73 mm, $P < 0.05$), approaching the optimal reproductive backfat range.

Table 4
Effect of Dietary Energy Level on First Estrus Parameters of Replacement Gilts

Item	T1	T2	T3	T4	T5	P-value
No. of gilts	72	70	71	72	70	
Age of gilts at first estrus, d	193.00 ± 16.67 ^{ab}	196.36 ± 18.93 ^a	191.37 ± 15.34 ^{ab}	186.78 ± 17.83 ^b	190.79 ± 21.59 ^{ab}	0.035
BW of gilts at first estrus, kg	125.33 ± 14.34	126.62 ± 16.17	125.15 ± 14.68	123.95 ± 16.21	125.15 ± 15.30	0.898
BF of gilts at first estrus, mm	11.94 ± 1.67	12.09 ± 1.47	12.23 ± 1.64	12.44 ± 1.80	12.51 ± 1.65	0.204
BW= body weight; BF = backfat thickness. The data in the table are presented as mean ± standard deviation; Different lowercase letters (a, b) as superscripts within the same row indicate significant differences among groups ($P < 0.05$).						

Table 5
Effect of Dietary Energy Level on First Mating Parameters of Replacement Gilts

Item	T1	T2	T3	T4	T5	P-value
No. of gilts	66	63	65	62	67	
Age of gilts at first mating, d	221.77 ± 10.88	224.32 ± 11.84	220.55 ± 11.26	220.18 ± 13.87	221.90 ± 13.84	0.367
BW of gilts at first mating, kg	146.41 ± 9.15	148.13 ± 13.29	148.98 ± 11.32	150.77 ± 10.55	150.02 ± 8.04	0.160
BF of gilts at first mating, mm	13.86 ± 1.26 ^b	13.73 ± 1.39 ^b	14.19 ± 1.56 ^{ab}	14.52 ± 1.43 ^a	14.19 ± 1.03 ^{ab}	0.010
No. of estruses at first mating	3.79 ± 0.12 ^a	3.64 ± 0.14 ^{ab}	3.56 ± 0.23 ^{ab}	3.35 ± 0.18 ^{ab}	3.24 ± 0.21 ^b	0.032
BW= body weight; BF = backfat thickness. The data in the table are presented as mean ± standard deviation; Different lowercase letters (a, b) as superscripts within the same row indicate significant differences among groups ($P < 0.05$).						

Estrus rate

As shown in Table 6, the effects of dietary NE levels on the estrus rate of gilts before 200 and 220 days of age. The estrus rate of replacement gilts before 200 days of age was significantly higher in Group T4 (81.94%,) than in Group T1(65.75%), and T2 (59.72%) ($P = 0.05$), with no significant differences observed compared to T3 (70.67%) and T5 (72.97%). Similarly, for the estrus rate before 220 days of age, T4 (94.44%) and T1(94.52%) were significantly higher than T5 (81.08%), but showed no significant difference compared with T2 (84.72%) and T3(89.33%).

Table 6
Effects of Dietary Net Energy Level on Estrus Rate in replacement Gilts

Item	T1	T2	T3	T4	T5	P Value
No. of gilts	73	72	75	72	74	
No. of gilts that exhibited estrus before 200 days of age	48	43	53	59	54	
No. of gilts that exhibited estrus before 220 days of age	69	61	67	68	60	
Estrus rate before 200 days of age, %	65.75 ^b	59.72 ^b	70.67 ^{ab}	81.94 ^a	72.97 ^{ab}	0.050
Estrus rate before 220 days of age, %	94.52 ^a	84.72 ^{ab}	89.33 ^{ab}	94.44 ^a	81.08 ^b	0.035
The data in the table are presented as mean $\pm$ standard deviation; Different lowercase letters (a, b,) as superscripts within the same row indicate significant differences among groups ($P < 0.05$).						

Reproductive hormone

As shown in Table 7, At single time point, serum E₂ concentration in T4 was lower than T2 ($P < 0.05$), while LH, FSH, insulin, leptin and IGF-1 showed no statistical difference ($P > 0.05$). During the 6-h dynamic sampling on day 17 after first estrus, serum LH and FSH levels in T4 were significantly higher than T2 (Fig. 1). Serum E₂ in T4 remained numerically lower across all time points without statistical difference.

Table 7
Effects of Dietary Net Energy Level on reproductive hormones in replacement Gilts

Item	T2	T4	P Value
No. of gilts	6	6	
LH, mIU/ml	123.16 $\pm$ 18.76	138.68 $\pm$ 10.53	0.108
FSH, mIU/ml	64.37 $\pm$ 14.76	52.98 $\pm$ 14.54	0.208
E2, mIU/ml	154.54 $\pm$ 49.60	99.30 $\pm$ 27.06	0.038
INS, mIU/ml	22.83 $\pm$ 6.57	23.31 $\pm$ 4.92	0.888
Leptin, mIU/ml	14.84 $\pm$ 3.16	17.26 $\pm$ 3.45	0.238
IGF-1, mIU/ml	154.67 $\pm$ 26.49	160.55 $\pm$ 18.59	0.667
LH = Luteinizing Hormone; FSH = Follicle-Stimulating Hormone; E2 = Estradiol; INS = Insulin; IGF-1 = Insulin - like Growth Factor - 1. The data in the table are presented as mean $\pm$ standard deviation.			

Metabolomics analysis

- In this study, non-targeted metabolomics was performed to compare metabolic profiles between the T2 and T4 groups (T2 vs T4). Differential metabolites were identified by a combination of multivariate and univariate statistical analyses. PCA revealed that PC1, PC2 and PC3 accounted for 11.9%, 11.09% and 8.33% of the total variance (Fig. 2A), respectively, with distinct separation between the two groups, suggesting that different treatments caused systematic alterations in the global metabolic profile. Volcano plot profiling identified 107 differentially expressed serum metabolites between the T4 and T2 groups. Specifically, 35 metabolites were significantly increased and 72 were decreased in T4, while 2304 metabolites exhibited no obvious differences in abundance (Fig. 2B). The clustering heatmap with Z-score normalization and metabolite classification demonstrated that the differential metabolites were mainly distributed in glycerophospholipids (PC, PE, PI), triglycerides (TG), oxidized lipids, lysophosphatidic acid (LPA), organic acids and derivatives, phenolics, heterocyclic compounds, benzene derivatives, small peptides, amines, and nucleotide metabolites, as well as several drug-related and plant-derived metabolites (Fig. 2C). The top differential metabolites ranked by fold change (FC) included Condurangenin C, Glyoxylic acid, PE - NMe₂(14:0/20:2(11Z,14Z)), 3-Hydroxyisovaleric acid, and p-Coumaraldehyde (Fig. 2D). These results indicated that lipid metabolism remodeling, organic acid metabolism, and small peptide metabolism represent the primary differential metabolic pathways between T4 and T2 groups, providing critical data support for subsequent mechanistic exploration and biomarker identification.

Metagenomic analysis

We performed metagenomic sequencing to compare the composition, diversity and functional profiles of gut microbiota between groups T2 and T4. No significant differences were found in species richness (Chao1 index, $P = 0.48$) and alpha diversity (Shannon index, $P = 0.25$) (Fig. 3A). Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarities showed that PCoA1 explained 43.84% of the total variance. Obvious segregation between the two groups confirmed divergent microbial community structures ($R^2 = 0.119$, $Pr = 0.026$) (Fig. 3B).

Genus-level analysis revealed high diversity of intestinal bacteria, with *Prevotella*, *Streptococcus*, *Clostridium* and *Treponema* identified as the predominant genera. The relative abundance of *Streptococcus* was significantly higher in sows of T4 than in T2 (Fig. 3C). At the species level (Fig. 3D), the majority of microbial taxa were unclassified or uncultivated, and merely ~ 25% could be taxonomically assigned to the species level. Among the annotated species, *Treponema bryantii*, *Lactobacillus johnsonii*, *Clostridiales bacterium* and *Streptococcus gallolyticus* were dominant.

Linear discriminant analysis effect size (LEfSe) with an LDA score threshold > 3 was used to identify group-specific discriminatory taxa (Fig. 3E). The genus *g_Streptococcus* was the most highly enriched taxon and yielded the maximum LDA score in T4. At the species level, *s_Streptococcus gallolyticus*, *s_Streptococcus equinus* and *s_Prevotella_sp_CAG279* were significantly overrepresented in T4. Conversely, T2 harbored a distinct set of microbial biomarkers. The taxa *o_Fibrobacterales*, *c_Fibrobacteria* and *g_Bacteroides* served as core biomarkers at the genus and higher taxonomic ranks, while *s_Lactobacillus amylovorus*, *s_Hallerella succinigenes*, *s_Prevotella_sp_P5_92* and *s_Prevotella_sp_P2_180* exhibited greater relative abundances in T2.

Metagenome-based KEGG pathway annotation was applied to predict microbial functions. Metastats analysis identified substantial functional divergence across multiple metabolic pathways between T2 and T4 (Fig. 3F). For carbohydrate and energy metabolism, the pathways of starch and sucrose metabolism ($P = 0.045$) and galactose metabolism ($P = 0.047$) were significantly overrepresented in T4 microbiota, which enhanced the capacity for

carbohydrate catabolism and dietary energy capture. The ABC transporters pathway was also markedly enriched in T4 ($P = 0.016$), suggesting strengthened active translocation of sugars, amino acids and other essential nutrients by intestinal bacteria. Notable differences were also observed in the biosynthesis of metabolites and cofactors. Multiple biosynthetic pathways closely associated with energy metabolism were strongly activated in T4, including folate biosynthesis ($P = 0.041$), aromatic amino acid (phenylalanine, tyrosine and tryptophan) biosynthesis ($P = 0.032$), and arginine biosynthesis ($P = 0.045$). Elevated production of these indispensable amino acids and vitamins not only promoted microbial growth, but may also modulate host physiological functions via microbial metabolites. In comparison, T2 exhibited higher enrichment in glycerolipid metabolism ($P = 0.008$) and nicotinate and nicotinamide metabolism ($P = 0.033$). This functional discrepancy indicates that gut microbiota adopt distinct adaptive strategies to maintain energy storage and redox homeostasis in response to different experimental interventions.

Integrated correlation analysis

To explore the intrinsic association between gut microbial shifts and host serum metabolome alterations, we conducted correlation analysis of differential microbial species and metabolites across T2 and T4 (Fig. 4).

Three T4-enriched taxa, namely *s__Streptococcus gallolyticus*, *s__Streptococcus equinus* and *s__Prevotella_sp_CAG279*, were strongly and positively correlated with a panel of upregulated organic acids and phospholipids. Significant positive correlations were observed between these microbes and glyoxylic acid, 3-hydroxyisovaleric acid and 1-palmitoylglycerol-3-phosphate (LPA) ($r > 0.4$). The expansion of these *Streptococcus* members contributed to the accumulation of energy metabolic intermediates and reproduction-associated bioactive lipids in the host.

Conversely, T2-enriched species including *s__Hallerella succinigenes*, *s__Prevotella_sp_P2.180* and *s__Prevotella_sp_P5.92* were significantly negatively correlated with the aforementioned metabolites.

The heatmap delineated a distinct microbe-metabolite network. The *Streptococcus gallolyticus*-centered microbiota in T4 regulated organic acid metabolism and lipid signal transduction, ultimately forming a high-energy metabolic status that benefited physiological development of replacement sows. This work offers solid molecular evidence for the mechanism by which high-energy diets improve sow reproductive performance through gut microbiota modulation.

Reproductive performance, farrowing rates and retention Rate

The effects of dietary NE levels on the reproductive performance of replacement gilts in their first two parities are presented in Table 8. There were no significant differences among treatment groups regarding average TNB, NBA, and NW per litter in the first two parities. Similarly, there were no significant differences among the treatment groups in the farrowing rates and retention rates of replacement gilts for the first and second parities (Table 9).

Table 8

Effects of Dietary Net Energy Levels on Reproductive Performance of Replacement Gilts in Their First Two Parities

Item	T1	T2	T3	T4	T5	P Value
No. of gilts	66	63	65	62	67	
TNB from first two parities per litter	22.88 ± 10.01	20.37 ± 11.16	21.74 ± 10.78	23.66 ± 9.61	22.90 ± 9.55	0.410
NBA from first two parities per litter	22.03 ± 9.80	20.05 ± 10.91	21.18 ± 10.77	23.03 ± 9.33	22.42 ± 9.41	0.501
NW from first two parities per litter	20.11 ± 8.46	18.44 ± 9.90	19.14 ± 9.53	20.32 ± 8.56	19.99 ± 9.39	0.761
TNB = total number of piglets born; NBA = number of live piglets; NW = number of weaned piglets. The data in the table are presented as mean ± standard deviation.						

Table 9

Effects of Dietary Net Energy Level on Farrowing Rate and Retention Rate of Replacement Gilts

Item	T1	T2	T3	T4	T5	P Value
No. of gilts mated for the first time	66	63	65	62	67	
No. of primiparous farrowings	61	53	56	58	60	
No. of post-weaning matings for primiparous sows	55	49	50	51	55	
No. of second farrowings	48	40	45	45	50	
No. of post-weaning matings for parity 2 sows	45	37	43	44	45	
Gilt-to-first-farrowing rate, %	92.4	84.1	86.2	93.5	89.6	0.375
Second farrowing rate, %	87.3	81.6	90.0	88.2	90.9	0.651
First parity retention rate, %	83.3	77.8	76.9	82.3	82.1	0.845
Second parity retention rate, %	68.2	58.7	66.2	71.0	67.2	0.672
The data in the table are presented as mean ± standard deviation						

Discussion

The present study systematically investigated the effects of graded dietary NE levels during the rearing period on growth performance, pubertal onset, reproductive hormones, estrus rate, and two-parity reproductive performance of Danish Landrace × Large White crossbred replacement gilts through a large-scale gradient feeding trial. More importantly, multi-omics integration elaborated the mechanistic basis of NE regulation on gilt reproductive development from metabolic and microecological perspectives, which is the novel highlight of this study. The findings provide critical empirical evidence for optimizing nutritional strategies tailored to the metabolic and reproductive characteristics of modern genetically improved gilts, addressing the existing mismatch between traditional feeding protocols and contemporary gilt biological potential.

Our data demonstrated that dietary NE significantly regulated ADG and F:G of replacement gilts. From trial initiation to 156 d of age and throughout the entire rearing period, ADG showed a linear increase and convex quadratic

response, while F:G exhibited a linear decrease and concave quadratic response with increasing NE levels. Notably, the T4 group (phase 1: 2,360 kcal/kg NE; phase 2: 2,310 kcal/kg NE) achieved optimal growth performance, with ADG significantly higher than that of T1, T2, and T3, and F:G significantly lower than those groups. This aligns with the core role of energy as a “nutritional nexus” in gilt developmental biology [20–21], as adequate NE supply promotes efficient metabolic partitioning of nutrients toward somatic growth, thereby improving growth rate and feed conversion efficiency. Interestingly, the T5 group (highest NE level) did not further enhance growth performance compared with T4, suggesting a threshold effect of dietary NE on gilt growth. Excessive energy intake may not continue to improve growth efficiency; instead, it may lead to metabolic redundancy or altered nutrient allocation patterns, consistent with previous observations that supra-optimal energy levels do not proportionally increase animal productivity [4]. Importantly, the absence of significant differences in body weight at puberty across treatments, despite variations in ADG and pubertal age, suggests that gilts across energy regimens ultimately reached a similar metabolic weight threshold necessary for HPO axis activation. This supports the concept of a critical body weight or body composition for puberty, as postulated by Patterson and Foxcroft (2019) [22].

Pubertal onset is a key milestone in gilt reproductive development, and its timing is closely linked to lifetime reproductive performance [22]. The most pronounced effect of dietary energy was on the timing of puberty. Gilts receiving moderate to high NE levels (T4, T5) attained puberty approximately 6–8 d earlier than those on lower energy regimens (T1, T2), while BW and BF at puberty were not affected by NE levels. This indicates that dietary NE primarily regulates pubertal timing through metabolic signaling rather than simply promoting body size or fat deposition, which aligns with the mechanism that dynamically altered energy homeostasis triggers hierarchical reallocation of metabolic resources, prioritizing reproductive programming when energy is sufficient [23].

The estrus rate, serving as a practical indicator of the synchronicity in reproductive development, exhibited a similar trend. The T4 group had the highest estrus rate before 200 days of age (81.94%), significantly higher than T1 (65.75%) and T2 (59.72%), and T4 and T1 groups had significantly higher estrus rates before 220 days of age than T5. The superior estrus performance of the T4 group may be attributed to the optimal activation of the HPO axis by moderate NE levels. In contrast, the lower estrus rate of T5 before 220 days may be related to potential metabolic perturbations caused by excessive energy, such as altered insulin sensitivity or leptin signaling, which indirectly interfere with HPO axis function [24]. These results support the previous finding that gilts reaching puberty before 210 days of age have optimal reproductive potential [7], and further clarify that dietary NE levels of 2,360 kcal/kg (120–156 days) and 2310 kcal/kg (160 days to pre-breeding) are conducive to achieving early and synchronized puberty in modern Danish crossbred gilts.

Reproductive hormone profiles directly reflect HPO axis function. The study found that although the basal level of E2 in T4 was significantly lower than that in T2, the 6-h dynamic sampling revealed that T4 had significantly higher serum LH and FSH levels than T2 on day 17 of the first estrous cycle. LH and FSH are key regulators of follicular development and ovulation: LH pulses promote ovulation, while FSH stimulates follicular growth and maturation [19–20]. The elevated LH and FSH levels in T4 suggest that moderate NE levels enhance the secretory activity of the pituitary gland, thereby promoting follicular development and pubertal onset, which explains the earlier puberty and higher estrus rate in T4. In contrast, the lack of significant differences in insulin, IGF-1, and leptin levels among groups may be due to the fixed crude protein and SID lysine levels in the diet. These hormones are often involved in the interaction between energy and protein metabolism [12], and the constant protein supply may have buffered the regulatory effect of NE on their secretion. Additionally, the single-time-point sampling of non-estrous gilts over 220

d of age may not have captured the dynamic changes in hormone levels; more frequent sampling or long-term tracking is needed to clarify the hormone-mediated mechanism of energy regulation of gilt reproduction.

Consistent with farm management protocols (first mating at ≥ 220 d of age), dietary NE levels did not affect the age and BW at first mating, but significantly influenced BF at first mating: T4 had a significantly higher BF (14.52 mm) than T1 (13.86 mm) and T2 (13.73 mm), approaching the optimal BF range (14–15 mm) for gilt reproductive performance reported by Tan et al. (2023) [7]. Appropriate BF at first mating is crucial for maintaining reproductive performance and reducing culling rates, as it provides energy reserves for gestation and lactation [5]. The BF of T4 further confirms that moderate NE levels promote the deposition of appropriate body fat, laying a foundation for subsequent reproduction.

Beyond phenotypic performance, multi-omics analysis in this study provided novel mechanistic insights into NE-regulated reproductive development from metabolic and microecological perspectives. Serum metabolomics clearly distinguished metabolic profiles between low- and moderate-NE groups, with differential metabolites mainly involved in lipid and organic acid metabolism. Lipid derivatives act as critical signaling molecules regulating ovarian follicle development and HPO axis activation [25], while organic acid intermediates participate in energy status sensing and reproductive programming [26]. The rewired metabolic landscape in T4 provided a favorable physiological microenvironment for accelerating puberty, which is consistent with findings that energy-driven metabolic remodeling facilitates reproductive axis maturation in mammals [27].

Gut metagenomics further confirmed that dietary NE remodeled microbial community structure rather than altering alpha diversity. Moderate NE selectively enriched *Streptococcus spp.*, which have been reported to enhance host energy harvest by promoting carbohydrate degradation and short-chain fatty acid production [28]. The strengthened pathways of carbohydrate catabolism, nutrient transmembrane transport, and biosynthesis of amino acids and vitamins in T4 facilitated energy harvesting and host physiological modulation, in line with studies showing that gut microbiota-derived metabolites influence reproductive hormone secretion and ovarian function [29]. In contrast, low-NE gilts tended to activate energy storage and redox-related pathways, limiting reproductive maturation potential, which echoes previous reports on energy restriction-induced gut microbial shifts and delayed puberty [9].

Integrated microbe-metabolite correlation analysis constructed a clear regulatory cascade: moderate NE enriched characteristic intestinal microbiota, which modulated serum organic acid and phospholipid metabolism, enhanced pulsatile LH and FSH secretion, and finally promoted earlier and synchronized puberty onset. This gut microbiota–serum metabolism–HPO axis regulatory model is supported by emerging evidence that intestinal microbes affect hypothalamic-pituitary function via microbial metabolites such as short-chain fatty acids and bile acids [27, 30]. The *Streptococcus gallolyticus*–centered microbiota observed in T4 aligns with the concept that specific commensal bacteria can act as “metabolic regulators” of host reproductive development [31]. Collectively, this study proposes a dietary NE–gut microbiota–serum metabolism–HPO axis regulatory model, revealing the intrinsic molecular mechanism of nutritional reproductive regulation in replacement gilts.

Despite the clear benefits for pubertal onset, the absence of significant effects on TNB, NBA, NW, farrowing rate, or retention rate in the first two parities warrants careful interpretation. This indicates that once replacement gilts achieve puberty and are bred at a standardized age with adequate body condition, their reproductive performance in the initial parity tends to remain stable across varying pre-pubertal nutritional regimens, provided a minimum nutritional threshold is satisfied [32–33]. Furthermore, standardized management practices during gestation and lactation offsets the initial differences in reproductive development induced by dietary NE levels [10, 34]. These

observations reinforce the principle of phase-specific nutritional management, wherein the primary objective during gilt rearing is to secure a high entry rate of well-developed, cyclic gilts into the breeding herd, rather than directly programming litter size. the robust genetic potential of modern Danish hybrid replacement gilts may mask the subtle effects of dietary net energy on reproductive performance [12]. Consequently, necessitating a larger sample size or longer-term tracking (≥ 3 parities) to detect significant differences.

Conclusions

This study demonstrates that the optimal phase-feeding NE levels (2,360 kcal/kg at 120–156 d and 2,310 kcal/kg from 157 d to pre-breeding) significantly improve growth efficiency, advance puberty onset by 6–8 d, increase 200-d estrus rate to 81.94%, and optimize first-mating backfat thickness, without affecting reproductive performance in the first two parities. Moderate dietary NE remodels serum metabolic profiling mainly involving glycerophospholipid, triglyceride, organic acid and small peptide metabolism, providing metabolic foundation for puberty initiation. Optimal NE reshapes gut microbial community structure, enriches *Streptococcus gallolyticus* and *Streptococcus equinus*, and enhances microbial functions of carbohydrate degradation, nutrient transport, and amino acid/vitamin biosynthesis. Gut microbiota-mediated metabolic reprogramming activates HPO axis hormone secretion, forming a gut microbiota–host metabolism axis to regulate puberty onset and estrus synchrony. This study establishes a practical NE nutritional standard for modern replacement gilts and provides multi-omics mechanistic evidence for nutritional regulation of gilt reproductive development. Long-term trials covering more parities are required to evaluate the persistent effects on sow lifetime productivity.

Declarations

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Authors' contributions

JW: investigation, data curation, software, writing-original draft. JJT: project administration, resources. HQS: formal analysis, methodology. HDJ: data curation, methodology. HKW: conceptualization, formal analysis. JP: conceptualization, funding acquisition, supervision. YFZ: conceptualization, methodology, formal analysis, writing-review & editing.

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Data availability

Data will be provided upon request.

Ethics approval and consent to participate

All experimental protocols used in the animal experiment were approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University (approval number: HZAUSW-2023-0027).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

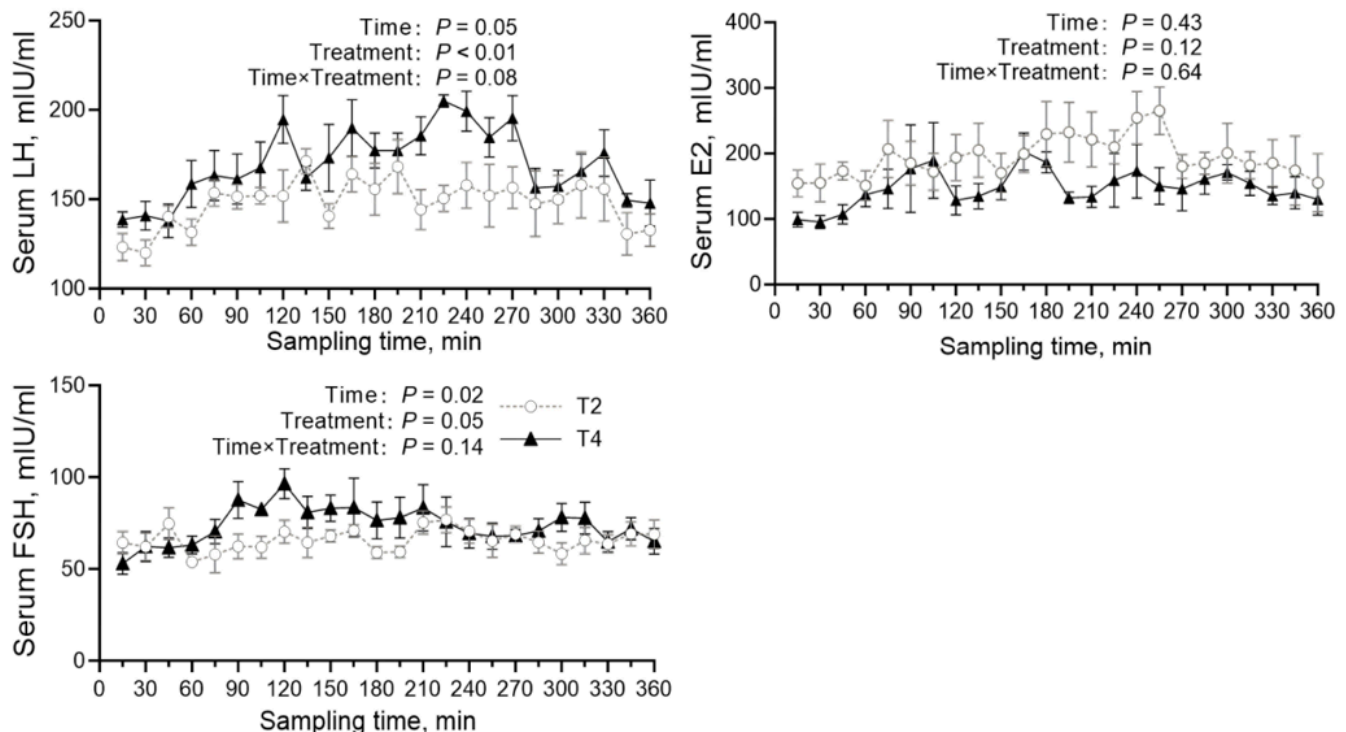


Figure 1

Changes in Reproductive Hormones of Replacement Gilts During a 6-Hour Period on Day 17 Post-First Estrus

LH = Luteinizing Hormone; FSH = Follicle-Stimulating Hormone; E2 = Estradiol

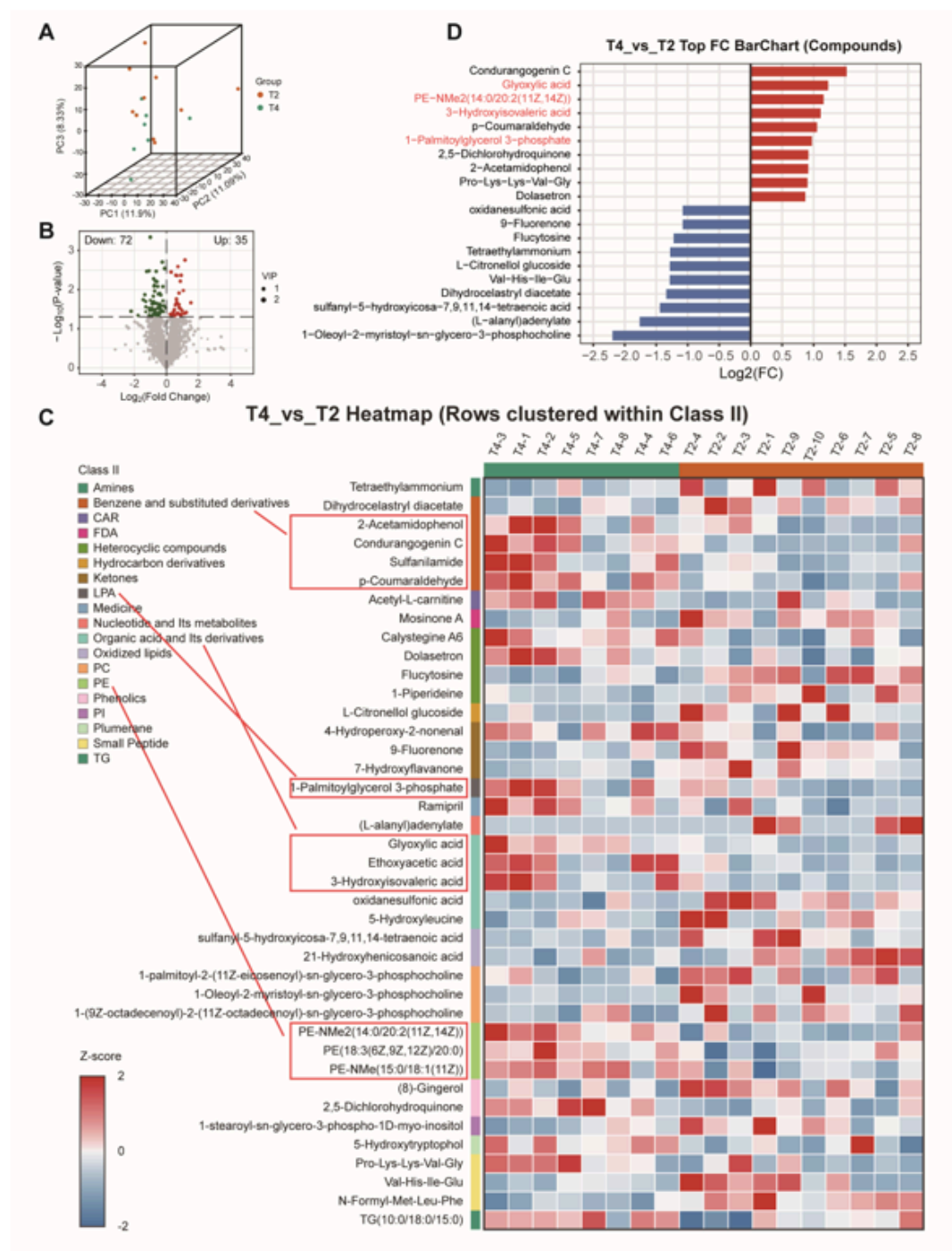


Figure 2

Integrated metabolomics analysis revealing metabolic profiling differences between T4 and T2 groups

(A) PCA score plot showing metabolic separation between T2 and T4. PC1 and PC3 explain 11.9% and 8.33% of total variance, respectively.

(B) Volcano plot illustrating 107 differential metabolites (35 upregulated, 72 downregulated) screened by Log₂FC, -Log₁₀(P-value), and VIP > 1.

(C) Z-score normalized hierarchical clustering heatmap of differential metabolites. Red indicates high abundance, blue indicates low abundance.

(D) Top differential metabolites ranked by fold change, including Condurangogenin C, Glyoxylic acid, PE-NMe₂(14:0/20:2(11Z,14Z)), 3-Hydroxyisovaleric acid, and p-Coumaraldehyde.

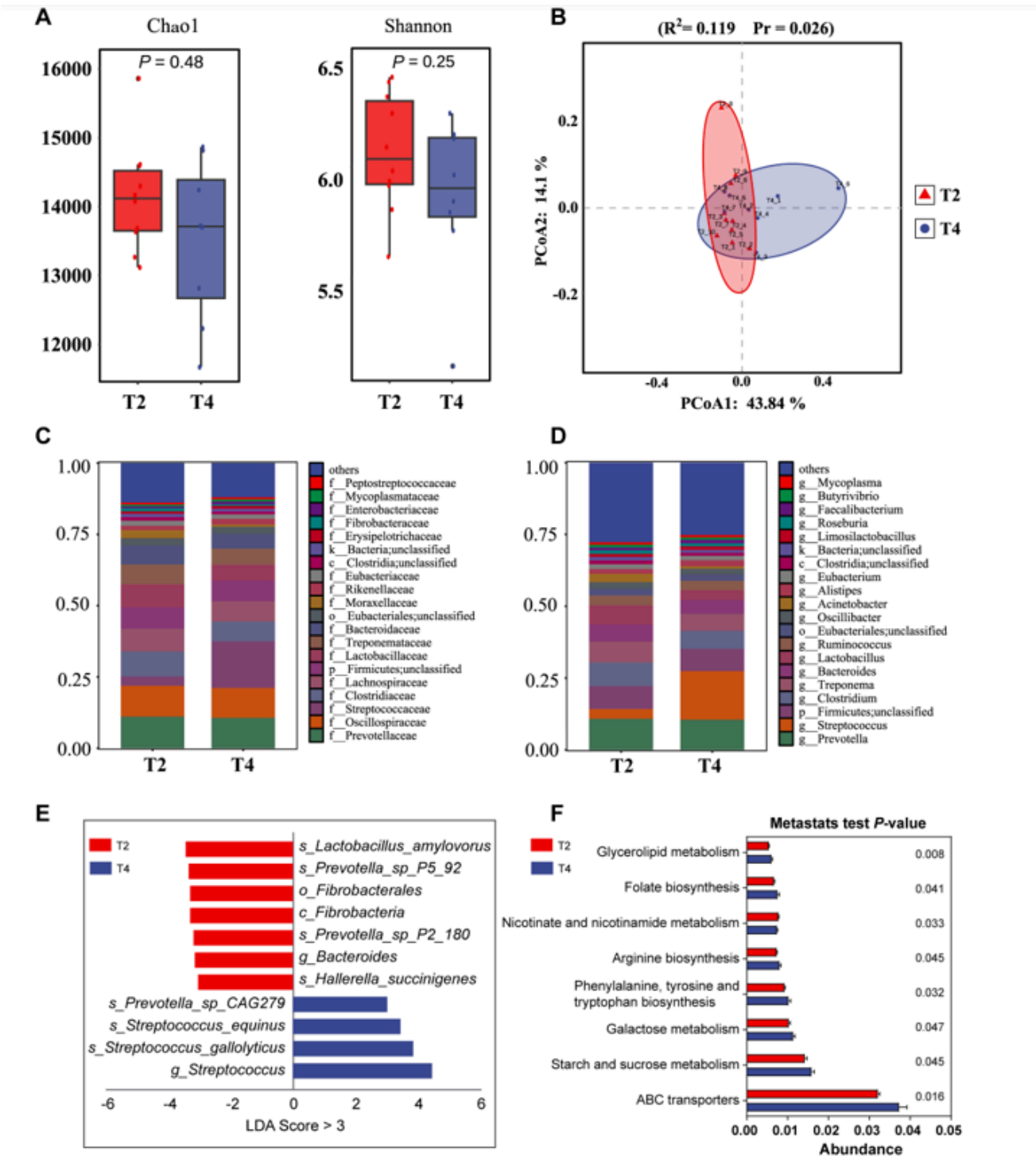


Figure 3

Microbial community diversity, taxonomic composition, and differential taxa analysis between T2 and T4 groups.

(A) α -diversity analysis indicated by Chao1 and Shannon indices. Shannon index showed significant differences between groups ($R^2=0.119$, $Pr=0.026$).

(B) β -diversity analysis based on Bray–Curtis distance using PCoA. PCoA1 explains 43.84% of the total variation, showing distinct separation of microbial community structure.

(C) Microbial taxonomic composition at the family levels.

(D) Microbial taxonomic composition at the and genus.

(E) LEfSe analysis identifying significantly differential taxa with LDA Score > 3 and $P < 0.05$.

(F) Differential microbial functional pathways between T2 and T4 groups based on KEGG enrichment analysis ($P < 0.05$).

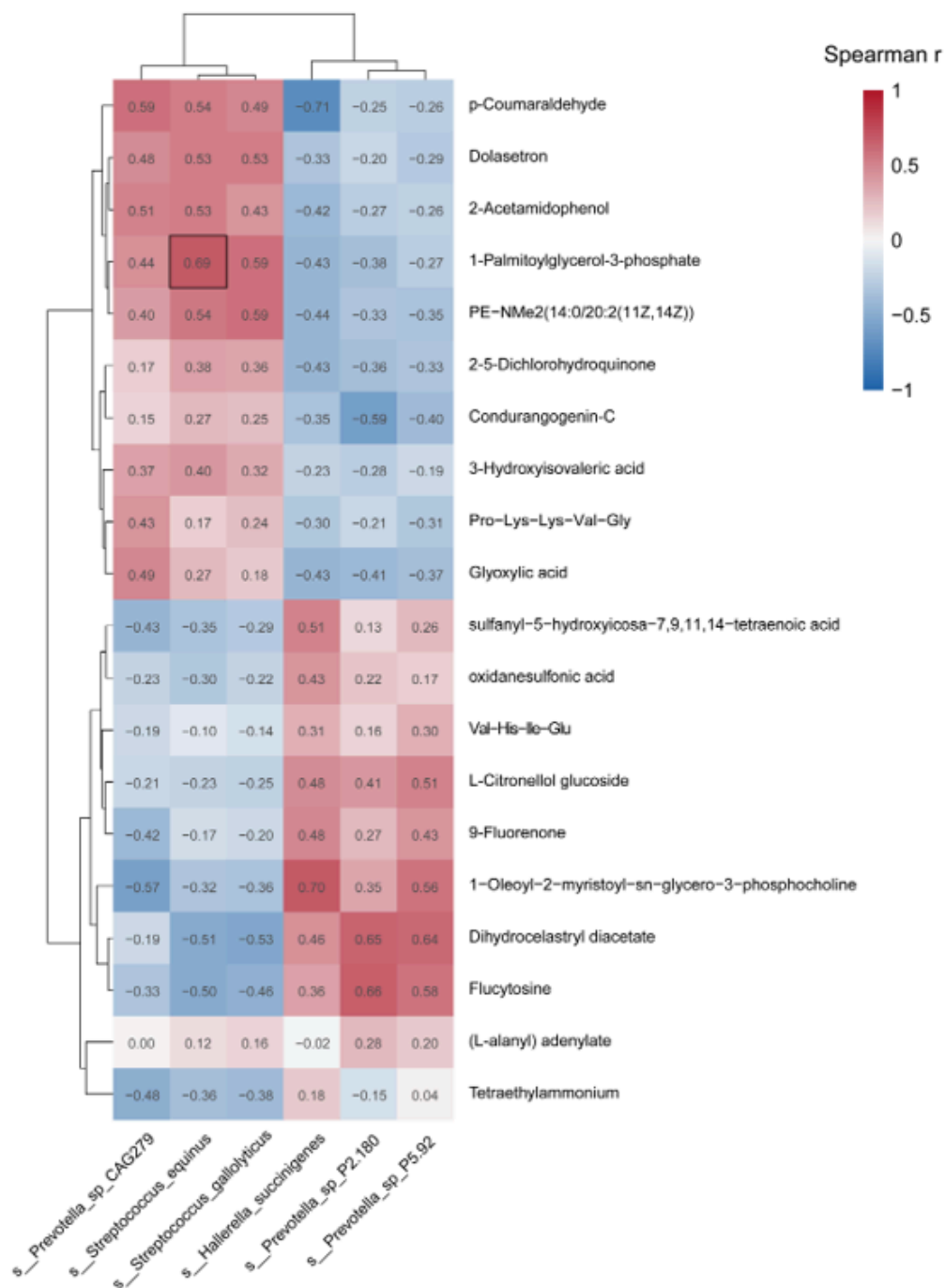


Figure 4

Microbial functional pathway enrichment and correlation analysis between metagenomics and metabolomics.

Spearman correlation heatmap between key differential microbial species and core differential metabolites. Red represents positive correlation and blue represents negative correlation; color intensity indicates the strength of the correlation coefficient (r).