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Persistent Benefits of Early Gestational Chenodeoxycholic Acid Supplementation on Late Pregnancy in Sows via Sustained Modulation of the Gut–Metabolism Axis

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A B S T R A C T

Background: Our previous studies in pigs have identified chenodeoxycholic acid (CDCA) as a potent metabolic regulator during early gestation, capable of enhancing embryo implantation by optimizing maternal metabolic status and gut microbiota–host interactions, whereas the long-term impacts of early gestational CDCA on late pregnancy remain largely unexplored.
Objectives: This study investigated whether supplementation with CDCA during early gestation (gestational day 0–28) exerts a sustained metabolic modulation effect on maternal health and reproductive outcomes in late gestation in a sow model.
Methods: Multiparous sows ($n = 24$) were randomly assigned to receive either a control diet or a diet supplemented with 0.15% CDCA during early gestation. The reproductive performance, oxidative stress, inflammatory status, gut microbiome, and serum metabolome in late gestation were subsequently evaluated.
Results: Results showed that early CDCA intervention significantly increased the total and live litter sizes ($P < 0.05$). Furthermore, CDCA treatment alleviated maternal oxidative stress and systemic inflammation and improved insulin sensitivity in late gestation ($P < 0.05$). Untargeted metabolomics revealed a distinct metabolic remodeling, characterized by the enrichment of beneficial metabolites, such as L-ornithine, and the depletion of proinflammatory and oxidative markers, including 1-palmitoylphosphatidylcholine, 2-lysophosphatidylcholine, 3-hydroxyanthranilic acid, and *N*-carboxymethyllysine. 16S rRNA sequencing indicated that CDCA exerted a targeted modulation rather than a global reconstruction of the gut microbiota. Specifically, CDCA suppressed potentially harmful bacteria, including *NK4A214_group*, *Clostridium_sensu_stricto_1*, and *Turicibacter*, whereas it enriched beneficial genera such as *Subdoligranulum*. Multiomics integration identified *NK4A214_group* as a key driver associated with exacerbated inflammatory status and unfavorable metabolic profiles.
Conclusions: Collectively, these findings demonstrate that early gestational CDCA supplementation elicits a long-term protective effect on maternal metabolism via optimizing specific gut microbe–metabolite interactions, thereby ensuring optimal gestational outcomes.

Keywords: chenodeoxycholic acid, early gestation, sow model, metabolic health, gut microbiota, metabolomics, reproductive performance

Abbreviations: CAT, catalase; CDCA, chenodeoxycholic acid; CML, *N*-carboxymethyllysine; CON, the control group; GLU, glucose; GSH-Px, glutathione peroxidase; 3-HAA, 3-hydroxyanthranilic acid; HOMA-IR, homeostasis model assessment of insulin resistance; IFN-γ, interferon-γ; INS, insulin; KEGG, Kyoto Encyclopedia of Genes and Genomes; LPC, lysophosphatidylcholine; MDA, malondialdehyde; NF-κB, Nuclear factor kappa B; OTU, operational taxonomic unit; PLS-DA, partial least squares discriminant analysis; 4PY, N1-methyl-4-pyridone-3-carboxamide; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TC, total cholesterol; VIP, Variable Importance in Projection.

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Introduction

Successful pregnancy relies on precise maternal metabolic adaptations. Global fertility rates have been gradually declining [1], with an estimated 15% of couples worldwide experiencing difficulties in achieving pregnancy [2]. Among various contributing factors, maternal metabolic disorders—often accompanied by oxidative stress and a proinflammatory state—are recognized as key contributors to impaired reproductive outcomes [3,4]. Between 2000 and 2023, the prevalence of metabolic syndrome in women rose from 14.7% to 31.0%, with ~846 million women affected globally in 2023 [5]. Therefore, effectively managing metabolic health during pregnancy to optimize reproductive outcomes represents an urgent public health priority. Owing to its substantial anatomical and physiological similarities to humans, the pig represents a highly relevant large animal model in gynecological research [6,7]. Consequently, the sow serves as an ideal translational model to investigate these complex metabolic processes and potential nutritional interventions during pregnancy.

Our previous research identified chenodeoxycholic acid (CDCA) as a potent "metabolic catalyzer" during early gestation [8]. CDCA supplementation was shown to enhance embryo implantation by optimizing ovarian progesterone synthesis, remodeling the maternal amino acid metabolism, improving anti-inflammatory and antioxidant capacity and insulin (INS) sensitivity, and, notably, the gut microbiota–host metabolite interaction network [8,9]. However, pregnancy is a continuous physiological process. As gestation advances, maternal systems face escalating metabolic demands, often manifesting as INS resistance and heightened oxidative stress and a low-grade inflammatory state [10–13]. Although these adaptations are physiologically necessary to support fetal development, their dysregulation can predispose mothers to gestational metabolic disorders, such as gestational diabetes and pre-eclampsia, and may also increase the risk of preterm birth and stillbirth, and adversely influence fetal programming [14–17]. Although nutritional intervention during late pregnancy has proven effective [18–20], it remains largely unexplored whether metabolic modulations applied strictly during early pregnancy can confer sustained benefits into late gestation. Our preliminary evidence indicates that the metabolic remodeling induced by early CDCA administration is not merely transiently restricted to the "implantation window" stage but may exert a persistent programming effect on maternal physiology throughout gestation [8]. Yet, whether this enduring influence depends on sustained modifications in the gut microbiota and its crosstalk with host metabolism remains to be fully elucidated.

To address these questions, we hypothesized that early gestational CDCA supplementation could induce long-term maternal metabolic programming, conferring sustained anti-inflammatory and antioxidant benefits into late gestation via the modulation of the gut microbiota–metabolome axis. To test this hypothesis, the present study evaluated the long-term impact of early gestational CDCA intervention on maternal metabolic health in late-pregnant sows. By integrating untargeted metabolomics and 16S rRNA sequencing, we aimed to *1*) determine whether the anti-inflammatory and antioxidant benefits of early CDCA supplementation persist into late gestation; *2*) elucidate the metabolic and microbial underpinnings of such sustained effects; and *3*) characterize the cooperative network linking the gut microbiota and host metabolites in late pregnancy, providing predictive insights into microbial functional adaptations. This investigation advances our understanding of bile acids as pivotal regulators of pregnancy-related metabolic programming and offers a conceptual framework for developing nutritional strategies aimed at "pregnancy health programming."

Methods

Animal experiment and sample collection

The study was approved by the Animal Welfare and Ethics Committee of the Beijing Academy of Agriculture and Forestry Sciences (approval number: IHVM11-2202-2). The experimental design and sample collection procedures followed the previous study with minor modifications [8]. A total of 24 healthy multiparous (Landrace × Large White) sows were selected and randomly allocated into 2 groups (n = 12) based on body weight, backfat thickness, parity, and previous litter size. In this study, the individual sow was considered the experimental unit. From the fourth day postweaning, estrus detection was performed twice daily using a teaser boar, and artificial insemination was conducted at the optimal breeding times, with a second insemination occurring 24 h later. The first day of insemination was designated as day 0 of gestation. From weaning to early gestation (days 0–28), the control group (CON) was fed a basal diet, whereas the treatment group received a basal diet supplemented with 0.15% CDCA. CDCA (catalog no.: S25542, ≥98% purity) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. The dose was selected based on our previous study [8]. For the remainder of the gestation period, both groups were provided with the same amount of gestation diet. Sows were managed under a restrictive feeding strategy to maintain appropriate body condition. Feeding quantities were regulated as follows: 2.5 kg/d premating, 2 kg/d during early gestation (days 0–28), 2.5 kg/d during midgestation (days 29–90), and 3 kg/d during late gestation (days 91 to parturition). The sows were housed individually with free access to water. All sows were housed in a uniform environment, with individual pen placement balanced between groups to minimize potential confounders. The basal diet met the nutrient requirements recommended by the National Research Council (2012) for pregnant sows [21], and dietary composition and nutrient concentrations are detailed in Table 1. Sows were examined for pregnancy via B-ultrasound at day 28 of gestation. Sows that failed to conceive or experienced abortion during the gestational period were excluded from the study, as maintaining pregnancy is a fundamental biological prerequisite for evaluating late-gestational metabolic adaptations and farrowing outcomes. Ultimately, 3 sows in the CON group and 1 sow in the CDCA group were excluded, resulting in a final sample size of n = 9 for the CON group and n = 11 for the CDCA group. On day 110 of gestation, following an overnight fast, 10 mL of blood was collected from the anterior vena cava prior to the morning feeding, centrifuged at 3000 × g for 15 min, and the serum was stored at −80°C for further analysis. To minimize analytical errors caused by sample conditions, 6 serum samples per group were randomly selected for subsequent biochemical and

TABLE 1
Ingredient composition of basal diet (as-fed basis)

Items	Estrus phase	Pregnancy phase
Corn	62.39	68.90
Soybean meal	18.50	14.50
Wheat bran	4.65	14.00
Fish meal	3.00	–
Extruded soybean	6.70	–
Monocalcium phosphate	0.95	0.40
Limestone	1.15	1.30
Soybean oil	1.00	–
Salt	0.30	0.40
Sodium bicarbonate	0.10	–
Sodium sulfate	0.10	–
Magnesium sulfate	0.20	–
Potassium chloride	0.15	–
L-Lysine HCl	0.35	0.22
L-Threonine	0.05	0.04
Choline chloride (60%)	0.10	–
Phytase	–	0.02
Vitamin-mineral premix	0.31	0.23

metabolomics analyses. Additionally, fresh fecal samples were collected from the rectum of each sow on day 110 of gestation and stored at −80°C for microbiota analysis. The numbers of total born, live born, stillborn, deformed, and mummified piglets were recorded at farrowing.

Determination of serum antioxidant, inflammatory, and INS sensitivity-related parameters

Oxidative stress-related indices, including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and catalase (CAT), inflammatory cytokines, including TNF-α, interferon-γ (IFN-γ), IL-1β, and IL-6, as well as metabolic parameters, including total cholesterol (TC), glucose (GLU), and INS in 6 randomly selected serum samples per group, were measured using specific commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. INS resistance was estimated using the homeostasis model assessment of INS resistance (HOMA-IR), calculated as INS (mIU/L) × GLU (mmol/L)/22.5.

Untargeted serum metabolomics analysis

Nontargeted metabolomics analysis of the 6 randomly selected serum samples per group was performed using a UHPLC–MS/MS system (n = 6 per group). Samples were prepared as follows: 100 μL of serum was mixed with 400 μL of extraction solution (acetonitrile:methanol = 1:1, v/v). The mixture was subjected to low-temperature ultrasonic extraction for 30 min (5°C, 40 kHz), followed by standing at −20°C for 30 min, and then centrifuged at 13,000 × g for 15 min at 4°C. The supernatant was collected, dried under nitrogen gas, and reconstituted in 120 μL of reconstitution solution (acetonitrile: water = 1:1, v/v). The reconstituted solution underwent a 5-min low-temperature ultrasonic extraction (5°C, 40 kHz), and the supernatant was collected into sample vials for analysis. A 3 μL aliquot of each sample was injected into an HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm). Additionally, to ensure analytical reliability, quality control (QC) samples were inserted at regular intervals. The mobile phases consisted of solvent A (water: acetonitrile = 95:5, v/v, containing 0.1% formic acid) and solvent B (acetonitrile:isopropanol:water = 47.5:47.5:5, v/v/v, containing 0.1% formic acid). The flow rate was set at 0.40 mL/min, and the column temperature was maintained at 40°C. The mass spectrometry conditions were as follows: The mass spectra were acquired in both positive and negative ion modes, with a mass scan range of 70 to 1050 m/z. The sheath gas flow rate was set to 50 Arb, the auxiliary gas flow rate to 13 Arb, and the auxiliary gas heater temperature to 425°C. The ion spray voltage was set at 3500 V for positive ions and −3500 V for negative ions. The ion transfer tube temperature was 325°C, and the collision energy was set to cycle through 20–40–60 V. The raw data were processed using the metabolomics software Progenesis QI (Waters Corporation), and the final data matrix was obtained for subsequent analyses. Both MS and MS/MS spectral information were annotated using public metabolite databases, including HMDB (http://www.hmdb.ca/) and Metlin (https://metlin.scripps.edu/) to identify the metabolites. The preprocessed data were then uploaded to the Majorbio Cloud platform (https://cloud.majorbio.com) for comprehensive data analysis. Next, based on the concentrations of metabolites in different samples, principal component analysis and partial least squares discriminant analysis (PLS-DA) analyses were conducted to evaluate the similarity of samples within groups and the differences between groups. Differential metabolites were identified as Variable Importance in Projection (VIP) >1 and P value <0.05. Functional Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the identified differential metabolites, and correlation analyses were carried out between these differential metabolites and relevant factors.

Gut microbiota DNA extraction, 16S rRNA sequencing, and data processing

Six fecal samples were randomly selected from each group for bacterial genomic DNA extraction, 16S rRNA sequencing, and data processing. Microbial genomic DNA from the feces was extracted using a commercial DNA kit (M5636; Omega Bio-tek), following the manufacturer's protocol. The purity and concentration of the extracted DNA were assessed using a NanoDrop 2000 spectrophotometer, and its quality was verified by agarose gel electrophoresis. The bacterial 16S rDNA V3 and V4 regions were amplified using specific universal primers (338F: 5′-ACTCCTACGGGAGGCAGCAG-3′, 806R: 5′-GGACTACHVGGGTWTCTAAT-3′), producing a fragment of ∼500 bp. The PCR products were recovered using 2% agarose gel electrophoresis and purified using the PCR Clean-Up Kit (YuHua). The purified products were quantified using a Qubit 4.0 (Thermo Fisher Scientific). Library construction was performed using the NEXTFLEX Rapid DNA-Seq Kit (Bioo Scientific). The constructed library was sequenced on the Illumina MiSeq PE300 platform (Illumina). QC of the raw paired-end sequencing reads was performed using fastp (version 0.19.6, https://github.com/OpenGene/fastp), and the reads were assembled using FLASH (version 1.2.11, http://www.cbcb.umd.edu/software/flash). The optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE (version 7.1) based on 97% similarity. Taxonomic annotation of OTUs was performed using the RDP classifier (version 2.11, http://rdp.cme.msu.edu/) based on the Silva 16S rRNA gene database (v138) with a 70%

confidence threshold. All data analyses were performed on the Majorbio Cloud platform, including α-and β-diversity indices, community composition and differences at various taxonomic levels, and correlation analyses between microbial genera and environmental factors. Functional prediction was conducted using PICRUSt2 (http://huttenhower.sph.harvard.edu/galaxy).

Data analysis and statistics

Data are presented as means ± SEM. All data were tested for outliers, normality, and homogeneity of variance prior to analysis. To accommodate the slight imbalance in final sample sizes between groups (CON, $n = 9$; CDCA, $n = 11$), data were analyzed using an unpaired 2-tailed Student's *t*-test performed in IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Visualizations were conducted using GraphPad Prism version 10.1.2 (GraphPad Software, Boston, MA, USA). The correlation analyses among the key parameters were conducted using Spearman's correlation analysis. Procrustes analysis was performed to assess the association between the microbiome and metabolome based on the R package vegan. Statistical significance was determined at $P < 0.05$, whereas $0.05 < P < 0.10$ was considered indicative of a trend.

Results

Early gestational dietary CDCA intervention improves the reproductive performance of sows

Table 2 shows that the total number of piglets and the number of live piglets were significantly higher in the CDCA group compared with the CON group ($P < 0.05$). No significant differences were observed in the numbers of stillborn, deformed, or mummified piglets ($P > 0.05$).

Early gestational dietary CDCA intervention alleviates oxidative stress, downregulates inflammatory responses, and improves GLU metabolism during late gestation

On day 110 of gestation, maternal serum concentrations of MDA were significantly reduced, whereas the activities of T-AOC, SOD, CAT, and GSH-Px were significantly increased in the CDCA group compared with CON (Figure 1, $P < 0.05$). Meanwhile, the concentrations of inflammatory cytokines, including IL-1β, IL-6, TNF-α, and IFN-γ, were significantly decreased ($P < 0.05$). In addition, INS concentrations were significantly reduced, with a decreasing trend observed in HOMA-IR ($0.05 \leq P \leq 0.10$), whereas no significant differences were observed in GLU or TC concentrations. These data indicated that early CDCA intervention exerts sustained anti-inflammatory and antioxidant effects in late gestation.

TABLE 2
Effects of CDCA supplementation on the reproductive performance of sows

Items	CON	CDCA	*P* value
Total born	14.00 ± 0.76	16.64 ± 0.79	0.029
Born live	12.78 ± 0.52	15.00 ± 0.80	0.040
Stillborn	0.89 ± 0.35	1.18 ± 0.26	0.412
Deformed	0.22 ± 0.22	0.27 ± 0.27	1.000
Mummified	0.11 ± 0.11	0.18 ± 0.12	0.824

Data are presented as means ± SEM. CON, control group fed a basal diet ($n = 9$); CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid ($n = 11$).
Abbreviations: CDCA, chenodeoxycholic acid; CON, control group.

Early gestational dietary CDCA intervention reshaped maternal metabolic profiles during late gestation

To systematically elucidate how early gestational CDCA intervention modulated maternal metabolic health, metabolic profiles of sows on day 110 of gestation were characterized. A PLS-DA revealed distinct separations between the CON and CDCA groups (Figure 2A–C), indicating significant metabolic reshaping. Furthermore, 37 significantly differential metabolites were identified. Among them, 11 metabolites were significantly upregulated and 26 metabolites were significantly downregulated in the CDCA group (Figure 2D). The top 30 differential metabolites based on VIP scores are presented in a heatmap (Figure 2E). Notably, the relative concentrations of metabolites, such as 2-lysophosphatidylcholine (2-LPC), *N*-carboxymethyllysine (CML), N1-methyl-4-pyridone-3-carboxamide (4PY), 1-palmitoylphosphatidylcholine, and 3-hydroxyanthranilic acid (3-HAA), were decreased, and concentrations of metabolites such as L-ornithine and alpha-bisabolol were increased in the CDCA group.

KEGG pathway enrichment analysis (Figure 2F) highlighted pathways related to amino acid and lipid metabolism, such as protein digestion and absorption, D-amino acid metabolism, alpha-linolenic acid metabolism, and choline metabolism pathways, and immune modulation-related pathways, such as the nuclear factor kappa B (NF-κB) signaling pathway, Th1/Th2/Th17 cell differentiation pathway, and T-cell/B-cell receptor signaling pathway, were significantly enriched. Furthermore, pathways related to cell growth or repair, such as growth hormone synthesis, mitogen-activated protein kinase, and ErbB signaling, were also enriched. Furthermore, KEGG topology analysis (Figure 2G) emphasized the centrality of amino acid metabolism (arginine biosynthesis, arginine and proline metabolism, glycine, serine, and threonine metabolism, and phenylalanine metabolism) and nicotinate and nicotinamide metabolism. These findings indicate that early CDCA intervention orchestrates systemic metabolic improvements involving nitrogen balance, immune regulation, and cellular processes.

Correlation analysis reveals favorable associations between serum metabolites and maternal oxidative stress, immune response, INS sensitivity, and reproductive performance

To further investigate whether the enhancement of maternal anti-inflammatory and antioxidant capacity is associated with alterations in the maternal metabolome, a correlation analysis was performed. Spearman's correlation analysis (Figure 3A) revealed that alpha-bisabolol, L-ornithine, and other 9 metabolites showed significant positive correlations with serum concentrations of GSH-Px, CAT, SOD, or T-AOC, or negative correlations with MDA concentrations, indicating these metabolites may contribute to combating oxidative stress. Moreover, these compounds were negatively correlated with inflammatory markers, such as IFN-γ, TNF-α, IL-6, or IL-1β, suggesting a close

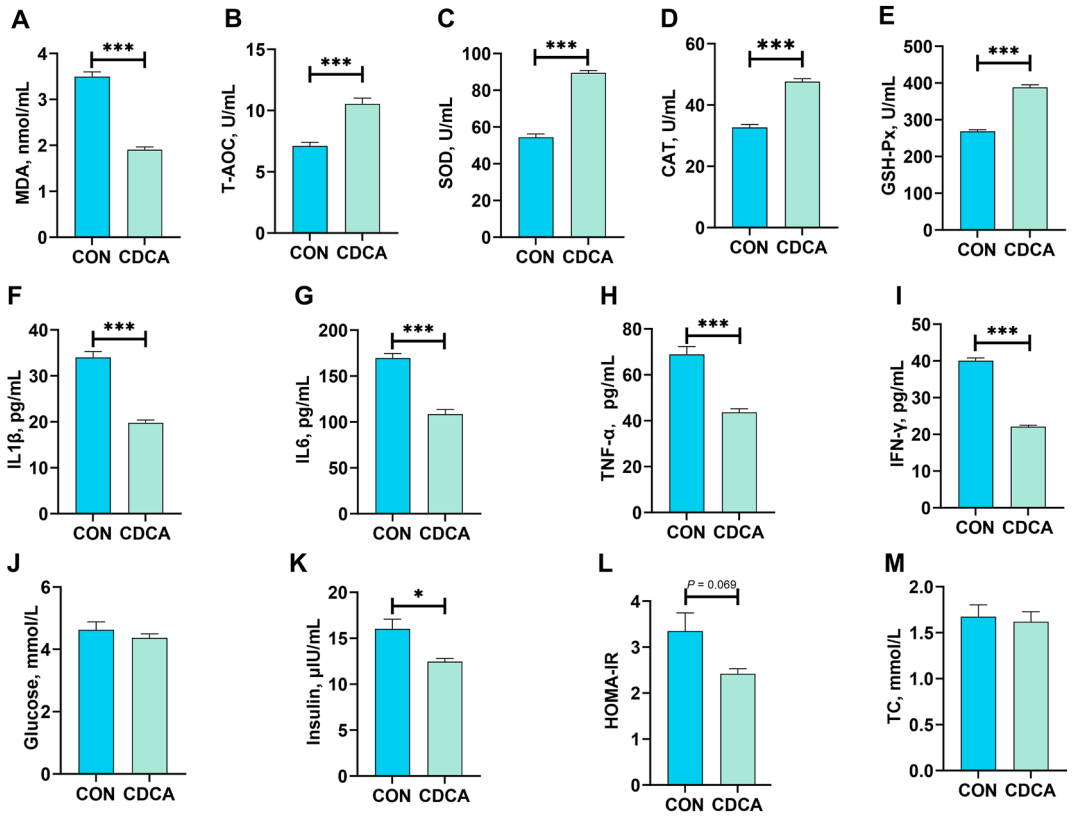

FIGURE 1. Effects of early gestational dietary CDCA intervention on oxidative stress, inflammatory responses, and glucose metabolism in sows on gestational day 110. Maternal serum concentrations of MDA (A), the activity of T-AOC (B), SOD (C), CAT (D), GSH-Px (E) were determined to evaluate oxidative status. Serum concentrations of inflammatory cytokines, including IL-1β (F), IL6 (G), TNF-α (H), and IFN-γ (I), were measured to assess systemic inflammation. Metabolic parameters, including glucose (J), insulin (K), HOMA-IR (L), and TC (M), were also analyzed. Data are presented as mean ± SEM ($n = 6$). CON, control group fed a basal diet; CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid. *$P < 0.05$, ***$P < 0.001$ between the control (CON) and CDCA groups. CAT, catalase; CDCA, chenodeoxycholic acid; CON, the control group; GLU, glucose; GSH-Px, glutathione peroxidase; HOMA-IR, homeostasis model assessment of insulin resistance; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; INS, insulin; MDA, malondialdehyde; SEM, standard error of the mean; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TC, total cholesterol; TNF-α, tumor necrosis factor-α.

association with reduced systemic inflammation. Conversely, 24 metabolites, including 4PY, 3-HAA, dodecanedioic acid, 1-palmitoylphosphatidylcholine, and 9(S)-hydroperoxy-octadecatrienoic acid (9(S)-HpOTrE), were positively correlated with oxidative stress and inflammation. Notably, 2-methylpropanamine exhibited significant negative correlations with INS concentrations and the HOMA-IR index, whereas 1,5-anhydro-D-glucitol positively correlated with INS concentrations.

Regarding reproductive performance (Figure 3B), 10 metabolites were positively related to the number of total piglets and live piglets, whereas 16 metabolites were negatively associated. No significant relationship exists between the different metabolites and the number of stillborn, mummified, and deformed. These results suggest that CDCA-induced metabolic remodeling promotes an antioxidant and anti-inflammatory profile conducive to optimal gestational outcomes.

Early gestational CDCA supplementation modulates specific microbes in sows during late gestation

To further analyze whether the positive regulatory effect of early CDCA intervention on late pregnancy was related to its modulation of gut microbiota, the gut microbiota in sows during late pregnancy was analyzed. Alpha diversity indices showed no significant differences between groups (Figure 4A–D), and principal coordinates analysis revealed similar community structures (Figure 4E). However, the Venn diagram identified 521 and 381 unique OTUs in the CON and CDCA groups, respectively (Figure 4F), indicating a certain number of differential microbes existing between the 2 groups.

Next, we analyzed the composition of the fecal microbiota. At the phylum level, the 4 dominant phyla, Firmicutes, Bacteroidota, Spirochaetota, and Proteobacteria, showed no significant differences (Figure 4G), although Fibrobacterota tended to be lower in the CDCA group ($P = 0.052$). The abundances of the top 20 microbial genera were pictured at the genus level, and the top 10 genera collectively constituted over 50% of the total microbiota (Figure 4H). At the genus level (Figure 4I), 23 microbial genera showed a differential trend ($P < 0.1$). Specifically, *NK4A214_group*, *norank_f_norank_o_Bradymonadales*, *Anaerofustis*, and *Anaeroplasma*, were significantly different between CON and CDCA groups ($P < 0.05$). The proportions of some genera, such as *Clostridium_sensu_stricto_1*, *NK4A214_group*, *Turicibacter*, *Butyricicoccus*, *Anaeroplasma*, were lower ($P < 0.1$),

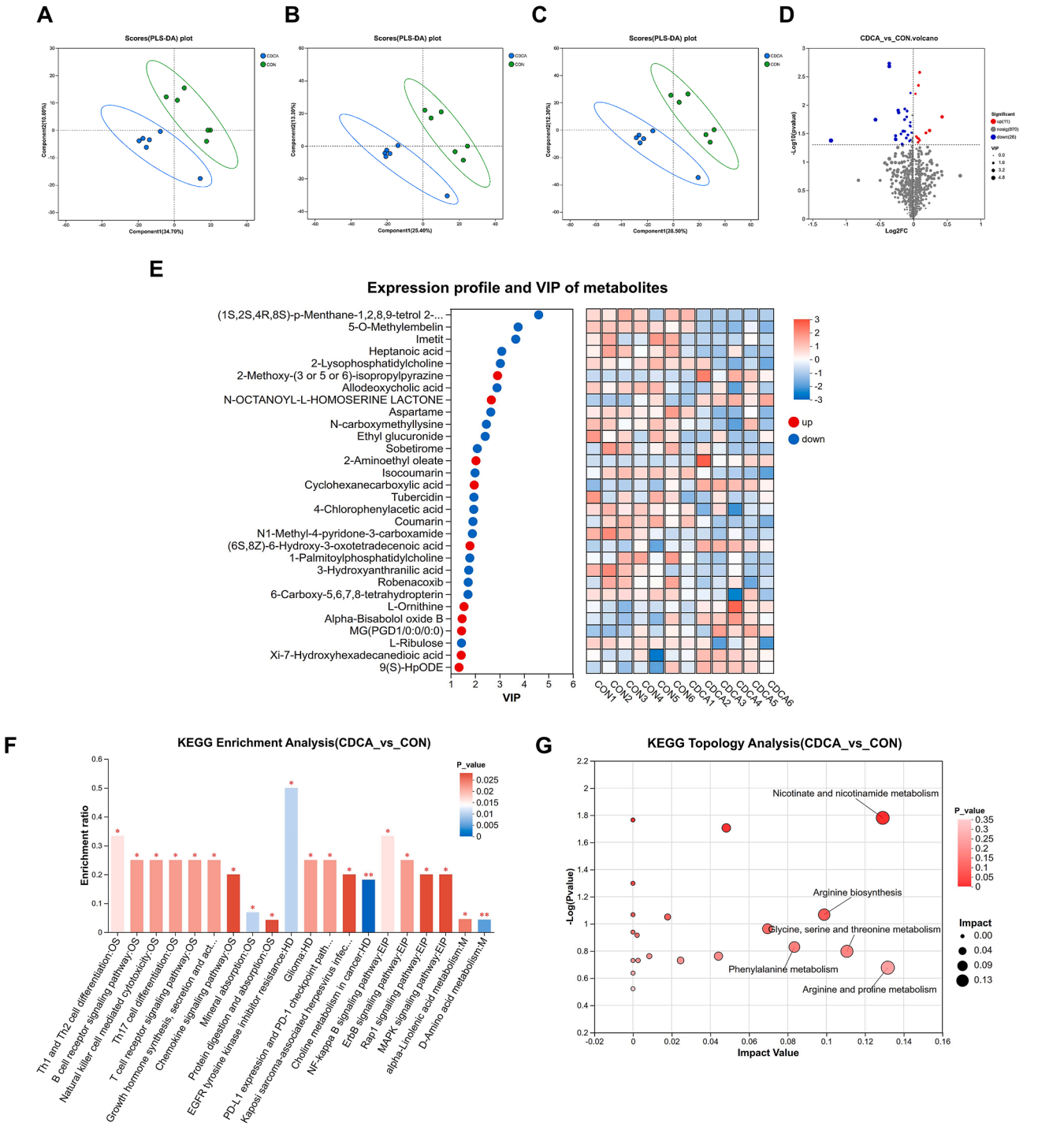

FIGURE 2. Effects of early gestational CDCA supplementation on the serum metabolomic profile of sows at day 110 of gestation. (A–C) PLS-DA score plots drive from (A) positive ion mode, (B) negative ion mode, and (C) mix mode. (D) Volcano plot of differential metabolites. Red dots represent significantly upregulated metabolites, blue dots represent significantly downregulated metabolites, and gray dots represent non-significant metabolites. (E) Hierarchical clustering heatmap and VIP scores of the top 30 differential metabolites. In the heatmap, red indicates higher relative abundance, while blue indicates lower abundance. A higher VIP value indicates a greater difference of the metabolite between the two groups. (F) KEGG pathway enrichment analysis. The x-axis represents the pathway name, and the y-axis represents the enrichment ratio, with a higher ratio indicating greater enrichment. The color of the bars indicates the significance of the enrichment. (G) KEGG topology analysis. Each bubble represents a KEGG pathway. The x-axis indicates the relative importance of metabolites within the pathway, and the y-axis represents the enrichment significance. The bubble size corresponds to the importance of the pathway. CON, control group fed a basal diet ($n = 6$); CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid ($n = 6$). $^*P < 0.05$, $^{**}P < 0.01$. CDCA, chenodeoxycholic acid; CON, the control group; KEGG, Kyoto Encyclopedia of Genes and Genomes; PLS-DA, partial least squares discriminant analysis; VIP, variable importance in projection.

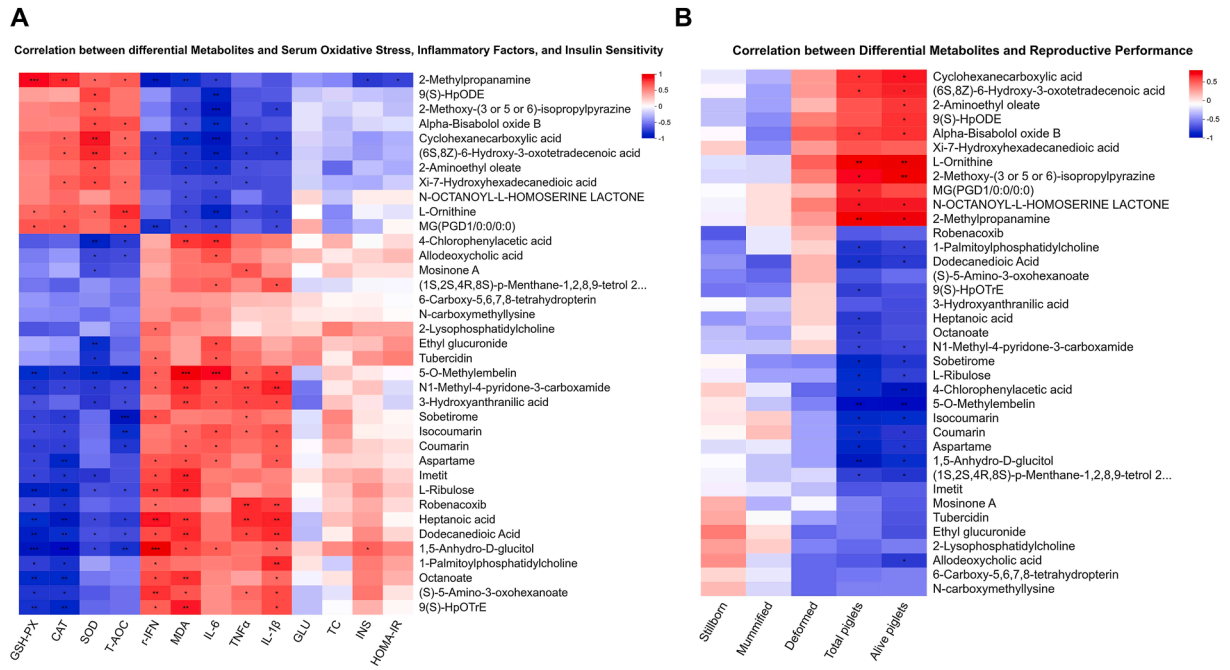

FIGURE 3. Association between serum metabolites and capacity of antioxidant, anti-inflammatory, insulin sensitivity, and reproductive performance of sows. Spearman correlation analysis between the total 37 differentially abundant serum metabolites at day 110 of gestation and serum oxidative stress, inflammatory response, insulin sensitivity (A), and reproductive performance (B). Red represents a positive correlation, while blue represents a negative correlation. CON, control group fed a basal diet ($n = 6$); CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$. CAT, catalase; CDCA, chenodeoxycholic acid; CON, the control group; GSH-Px, glutathione peroxidase; HOMA-IR, homeostasis model assessment of insulin resistance; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TNF-α, tumor necrosis factor-α.

whereas the proportions of other genera, such as *Subdoligranulum* and *Anaerofustis*, were higher in the CDCA group ($P < 0.1$). Collectively, CDCA supplementation did not alter the structure of the gut microbiota but did affect the abundance of some specific microbial genera.

Associations among gut microbiota, systemic metabolites, and maternal metabolic health indicators

To elucidate the functional changes corresponding to these microbial alterations, we carried out a functional prediction analysis using PICRUSt2. Results showed that maternal CDCA supplementation significantly downregulated microbial amino acid biosynthesis pathways (Figure 5A, $P < 0.05$). Consistently, serum metabolomics revealed decreased concentrations of amino acid derivatives (3-HAA, (S)-5-amino-3-oxohexanoate, and 4-chlorophenylacetic acid) and increased L-ornithine in the CDCA group (Figure 5B–E). Spearman's correlation heatmap revealed specific relationships between the differential microbes and differential metabolites (Figure 5F). For example, genera like *Anaerofustis*, *Eubacterium_eligens_group*, *k_norank_d_Bacteria*, *NK4A214_group*, and *norank_f_norank_o_Bradymonadales* showed significant correlations with multiple differential metabolites. Specifically, 4-chlorophenylacetic acid was negatively correlated with *Anaerofustis* and positively correlated with *Clostridium_sensu_stricto_1*. L-ornithine was positively correlated with *Anaerofustis*. 3-HAA was positively correlated with *NK4A214_group*. In addition, (S)-5-amino-3-oxohexanoate was positively correlated with *Turicibacter*. Although Procrustes analysis showed no significant global congruence between the gut microbiome and the maternal metabolome (Figure 5G, Monte Carlo P value = 0.284; M2 = 0.828), these specific associations suggest that CDCA modulates metabolic regulation via targeted microbial interactions.

We further correlated the top 30 most abundant microbial genera with key maternal health indicators (Figure 6A). *NK4A214_group*, significantly reduced by CDCA, was positively correlated with serum concentrations of all measured inflammatory cytokines (TNF-α, IFN-γ, IL-1β, and IL-6) and MDA, and negatively correlated with antioxidant enzymes, SOD and T-AOC. Similarly, the genus *UCG-005* concentrations were positively correlated with TNF-α and IL-6 concentrations and negatively correlated with SOD and T-AOC concentrations. The genus *Lachnospiraceae_XPB1014_group* was positively correlated with IL-1β, whereas *Clostridium_sensu_stricto_1* was positively correlated with IL-6 and negatively correlated with SOD concentrations. Regarding INS sensitivity, *Prevotella* and *Escherichia–Shigella* correlated positively with HOMA-IR, whereas *norank_f_norank_o_RF39* showed a negative correlation with INS and HOMA-IR. *Rikenellaceae_RC9_gut_group* and *Lachnospiraceae_NK4A136_group* were negatively correlated with GLU. Comprehensive analysis identified *NK4A214_group* as a key CDCA-driven microbe linked to improved metabolic status,

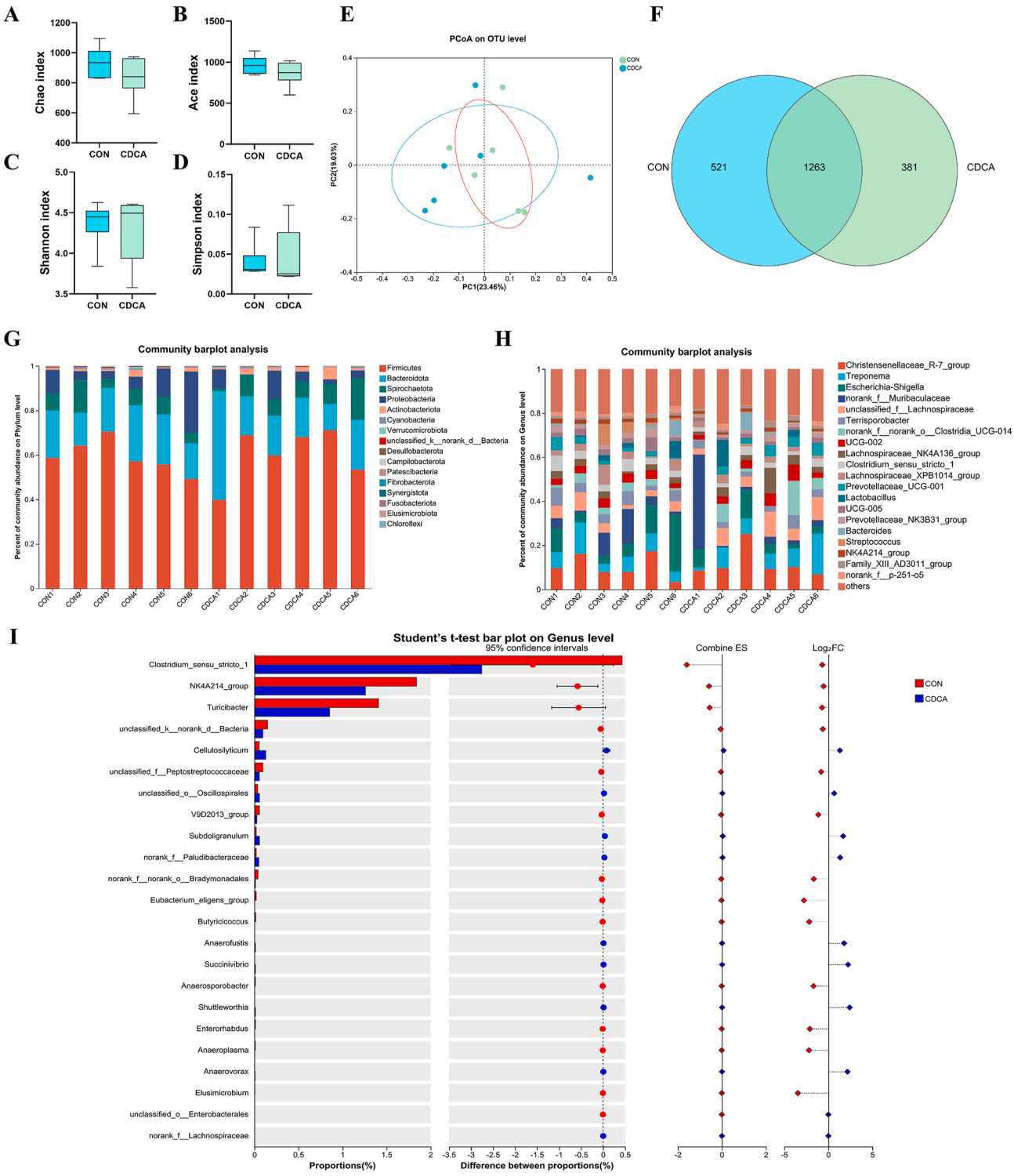

FIGURE 4. Effects of early gestational CDCA supplementation on fecal microbial diversity and composition in sows at day 110 of gestation. (A–D) Illustrates α-diversity indices of the gut microbiota, including (A) Chao index, (B) ACE index, (C) Shannon index, and (D) Simpson index. (E) Beta-diversity analysis (PCoA) comparing microbial community composition between the groups. (F) Venn diagram depicting shared and unique OTUs between groups. (G) Relative abundance of major microbial phyla in each fecal sample. (H) Relative proportions of the top 20 most abundant microbial genera. (I) Bar plot showing microbial genera with differential relative abundances between the 2 groups ($P < 0.1$). CON, control group fed a basal diet ($n = 6$); CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid ($n = 6$). CDCA, chenodeoxycholic acid; CON, the control group; OTU, operational taxonomic unit; PCoA, principal coordinate analysis.

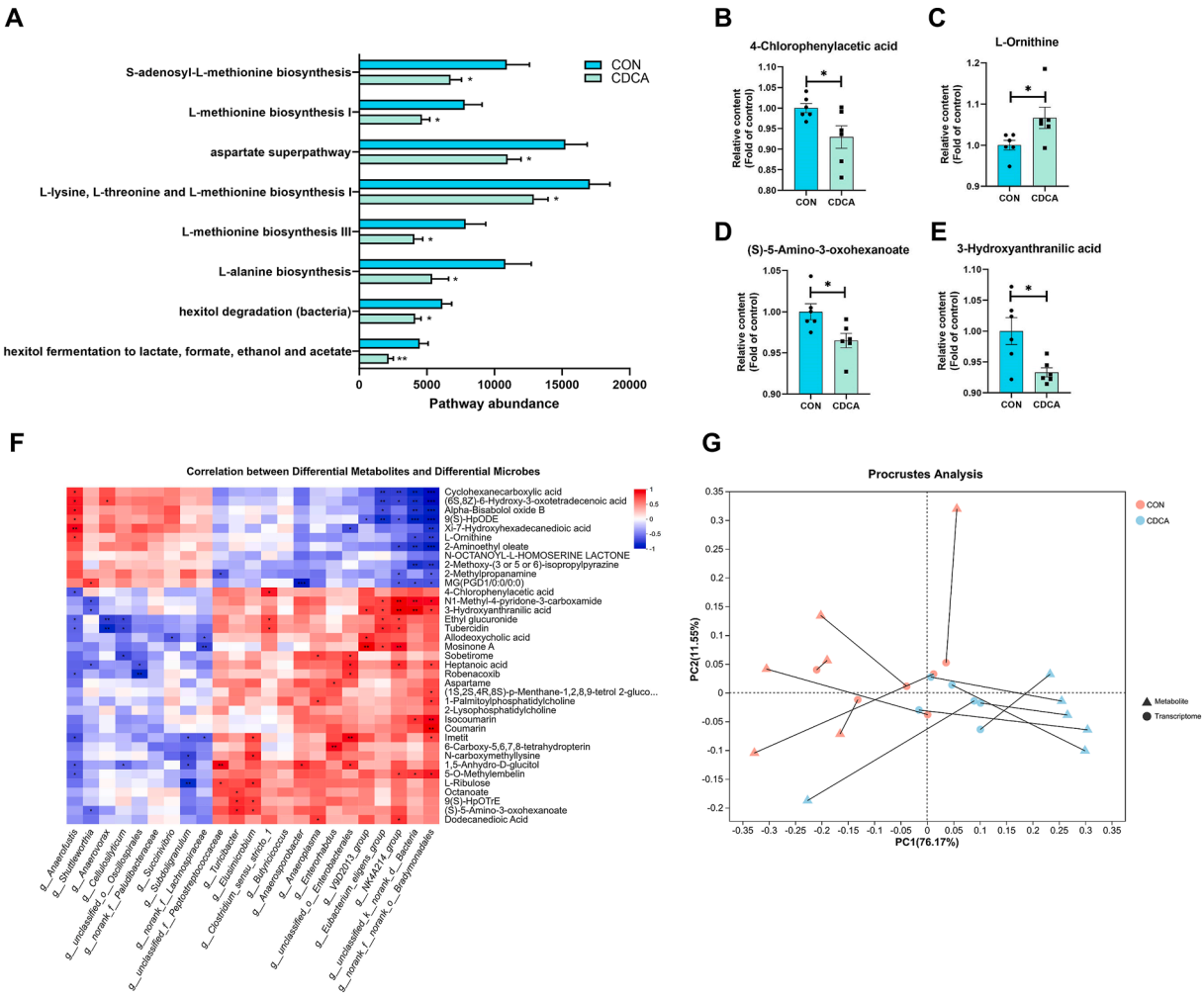

FIGURE 5. Relationship between specific gut microbiota and serum metabolic alterations in sows at day 110 of gestation. (A) PICRUSt2 functional prediction to infer the functional potential of microbial communities based on the MetaCyc database. (B–E) Relative concentrations of representative differential metabolites involved in amino acid metabolism, including (B) 4-Chlorophenylacetic acid, (C) L-Ornithine, (D) (S)-5-Amino-3-oxohexanoate, and (E) 3-Hydroxyanthranilic acid. (F) Spearman correlation heatmap between differential serum metabolites and differential microbial genera. Red indicates positive correlation, whereas blue indicates negative correlation. (G) Procrustes analysis showing the correlation between differential serum metabolites and differential fecal genera. CON, control group fed a basal diet; CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid. Data are presented as mean ± SEM ($n = 6$). *$P < 0.05$, **$P < 0.01$. CDCA, chenodeoxycholic acid; CON, the control group; PICRUSt2, Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2; SEM, standard error of the mean.

antioxidant capacity, anti-inflammatory activity, INS sensitivity, and reproductive performance of sows (Figure 6B–J).

Collectively, these results indicate that early pregnancy CDCA intervention modifies gut microbiota composition and function, driving favorable metabolic adaptations that persist into late gestation.

Discussion

Adverse maternal metabolic conditions during pregnancy are key contributors to declining reproductive capacity in mammals [22]. Although late-gestational nutritional interventions have shown promise, the persistent "programming" potential of early interventions remains understudied. Our previous work established CDCA as a metabolic regulator during early gestation [8, 9]. In the present study, we demonstrate that early CDCA supplementation confers sustained metabolic benefits that persist into late gestation. It is important to emphasize that although pregnancy is a dynamic immunological process requiring a baseline level of inflammation for normal progression, modern hyperprolific sows naturally experience metabolic syndrome during late gestation, which often leads to INS resistance, systemic oxidative stress, and a low-grade inflammatory state [23–25]. In the present study, the CDCA-driven reduction in MDA and proinflammatory cytokines and increase in antioxidant enzyme activities represent a mitigation of this metabolic burden rather than an inhibition of essential immune processes. The beneficial effects of CDCA-driven mitigation in maternal metabolic burden are directly validated by the significant improvement in reproductive performance, specifically the increase in the total number of piglets and live births. These findings confirm that the observed physiological shifts are

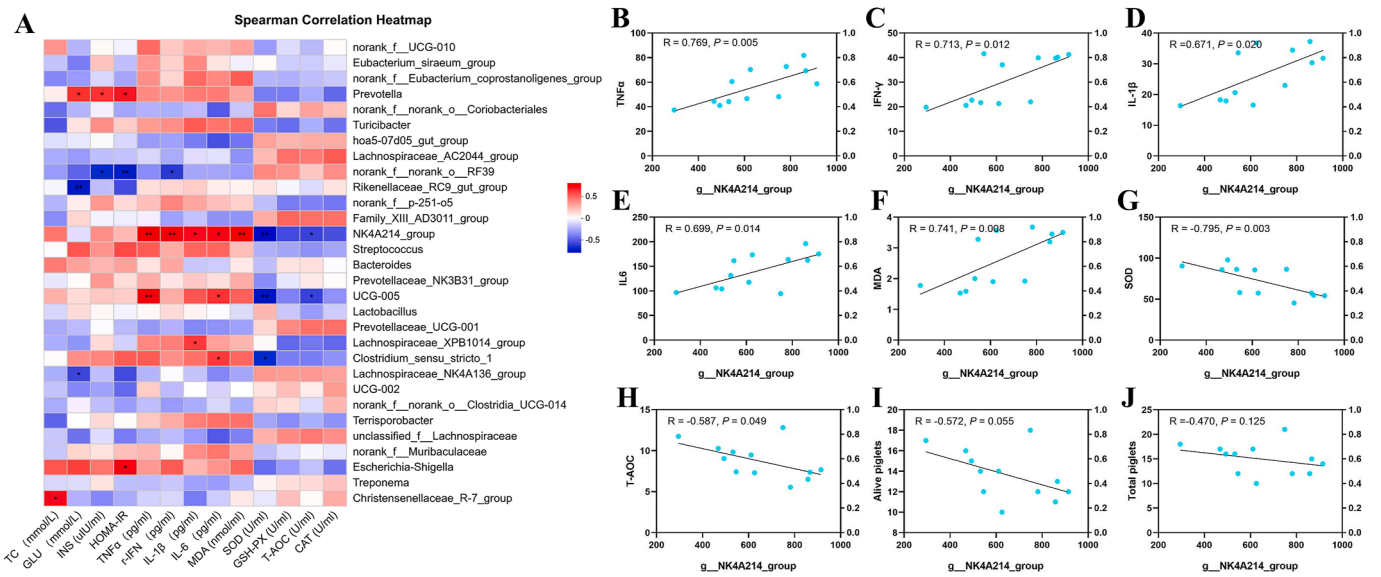

FIGURE 6. Associations between gut microbiota and serum insulin sensitivity, oxidative stress, and inflammatory markers. (A) Spearman correlation heatmap between the Top 30 most abundant intestinal microbial genera and serum insulin sensitivity, oxidative stress, and inflammatory markers in sows at Day 110 of gestation. Red grids represent positive correlations, whereas blue grids indicate negative correlations. (B–J) Scatter plots showing the linear correlations between the relative abundance of *g_NK4A214_group* and serum concentrations of TNF-α (B), IFN-γ(C), IL-1β (D), IL-6 (E), MDA (F), SOD (G), T-AOC (H), as well as reproductive performance indicators alive piglets (I), and total piglets (J). *R* values indicate the Spearman correlation coefficient, and *P* values indicate statistical significance. CON, control group fed a basal diet ($n = 6$); CDCA, treatment group fed a basal diet supplemented with 0.15% chenodeoxycholic acid ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$. CAT, catalase; CDCA, chenodeoxycholic acid; CON, the control group; GSH-Px, glutathione peroxidase; HOMA-IR, homeostasis model assessment of insulin resistance; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; MDA, malondialdehyde; SEM, standard error of the mean; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TNF-α, tumor necrosis factor-α.

indeed beneficial and underscore the critical window of early pregnancy for nutritional programming of maternal health [26].

CDCA treatment induced significant metabolic remodeling in sows, primarily clustered in lipid metabolism, amino acid metabolism, immune regulation, and cellular growth. We propose that these metabolic shifts are central to CDCA's regulatory effects on maternal health. Regarding the lipid metabolism, we observed a significant downregulation of LPC, including 1-palmitoylphosphatidylcholine and 2-LPC. Although LPCs serve as important carriers for the placental transfer of polyunsaturated fatty acids [27], they also function as potent proinflammatory mediators linked to oxidative stress [28] and INS resistance [29]. The reduction of LPCs, alongside the downregulation of lipid peroxidation biomarkers dodecanedioic acid [30] and the oxylipin 9(S)-HpOTrE, which correlated with inflammation response [31], indicates an alleviation of systemic metabolic stress and inflammatory status in CDCA-treated sows. Crucially, as the placenta possesses redundant transport mechanisms, this remodeling likely optimized the gestational environment without compromising essential nutrient delivery, which is supported by the significantly improved reproductive performance. Moreover, the upregulation of alpha-bisabolol and its derivatives further strengthened this protective profile through their potent anti-inflammatory and antioxidant properties [32, 33].

Second, CDCA intervention significantly modulated amino acid metabolism, particularly the arginine biosynthesis and the polyamine pathways, which are critical mechanisms for placental angiogenesis and fetal growth [34,35]. In the present study, arginine biosynthesis emerged as a key pathway, aligning with the specific upregulation of L-ornithine. As a central node linking the urea cycle and polyamine biosynthesis, the elevation of L-ornithine not only indicates improved ammonia detoxification but also provides precursors for polyamines, which are crucial for placental vascularization [36]. Furthermore, L-ornithine has been shown to exert anti-inflammatory effects [37] and correlates negatively with metabolic disorders [38]. This beneficial shift was paralleled by the depletion of harmful amino acid derivatives associated with systemic inflammation and INS resistance. Specifically, 3-HAA and CML were downregulated in CDCA. Both of them typically accumulate under inflammatory states [39,40] and trigger proinflammatory pathways like NF-κB [41]. Furthermore, elevated CML concentrations have been linked to the development of INS resistance [42]. Consequently, the observed reduction in CML indicates a suppressed inflammation status and mitigated metabolic burden. Additionally, the reduction in 4PY, a marker of vascular inflammation [43], further supports the protective effect of CDCA on maternal health. Collectively, these amino acid-related metabolic shifts provide a robust molecular basis for the enhanced reproductive performance observed in CDCA-treated sows.

Gut microbiota modulation appears to be a key mechanism underlying these sustained effects of early CDCA supplementation. This study found that CDCA addition during early gestation did not significantly affect the global diversity or phylum-level composition of the gut microbiota in late gestation, which is consistent with the observations in early gestation [8]. In addition, at the genus level, compared with our previous finding in early pregnancy, the top 20 abundant genera showed relatively stable compositions, and the 2 most abundant genera

remained *Christensenellaceae_R-7_group* and *Treponema* [8]. However, the microbial profile during late gestation was characterized by a dynamic enrichment of potential pathobionts. We observed that the proportions of *Escherichia–Shigella* and *Terrisporobacter* were higher than in early pregnancy. *Escherichia–Shigella* is typically associated with inflammation and INS insensitivity, and an increase in *Escherichia–Shigella* abundance in late-pregnancy sows can lead to elevated endotoxin concentrations, thereby exacerbating inflammation during late pregnancy in sows [44]. Similarly, *Terrisporobacter* is an anaerobic pathogen whose increase in abundance can lead to oxidative stress [45,46]. This proinflammatory shift in the microbial environment as pregnancy advances highlights the importance of targeted interventions to maintain maternal gut microbiota homeostasis.

Beyond gestational adaptations, CDCA supplementation significantly remodeled the gut microbiota by suppressing potential pathobionts and enriching beneficial bacteria. Regarding the specific impact of CDCA, *NK4A214_group*, *norank_f_norank_o_Bradymonadales*, *Anaeroplasma*, and *Anaerofustis* were the main differential genera between the CDCA and CON groups. Among the differential genera, *NK4A214_group* exhibited a strong positive correlation with maternal inflammatory response and a negative correlation with antioxidant enzyme concentrations. Notably, dietary *Clostridium butyricum* has been shown to improve immune responses and antioxidant capacity while concurrently reducing *NK4A214_group* abundance [47]. Although some studies suggest that a decrease in this genus may induce metabolic disorders and inflammatory diseases [48], our data indicate that under late-gestation metabolic stress, the enrichment of *NK4A214_group* is closely linked to an exacerbated inflammatory and oxidative stress. This aligns with network ecology analyses identifying this group as a key taxon associated with progression of INS resistance and type 2 diabetes [49]. Furthermore, CDCA treatment reduced the abundance of *Anaeroplasma*, a genus enriched in high-inflammatory models and positively correlated with intestinal inflammation biomarkers [50]. Other opportunistic pathogens, such as *Clostridium_sensu_stricto_1* and *Turicibacter*, both strongly associated with gut inflammation, barrier dysfunction, and proinflammatory cytokine elevation [51–54], were similarly reduced. Conversely, CDCA promoted the enrichment of *Subdoligranulum*, a key butyrate-producing bacterium associated with improved INS sensitivity, gut barrier integrity, and healthy metabolic status [55,56]. Collectively, these targeted microbial shifts, characterized by the suppression of harmful taxa and the promotion of beneficial bacteria, provide a robust biological basis for the observed improvements in maternal metabolic health.

Beyond compositional changes, the functional prediction of gut microbiota indicated a significant downregulation of pathways related to amino acid biosynthesis in the CDCA group. This was consistent with our serum metabolomics data, which showed reduced concentrations of potentially harmful amino acid-derived metabolites, such as 3-HAA and 4-chlorophenylacetic acid. This concordance suggests that CDCA remodels specific microbial functions to suppress the production of potentially harmful amino acid derivatives. Notably, although Procrustes analysis showed no significant global congruence, Spearman's correlation revealed strong, specific correlations between differential genera and metabolites, indicating that CDCA exerts a targeted modulation rather than a global reconstruction. The correlation heatmap further identified the *NK4A214_group*, which was significantly suppressed by CDCA, as a potential key driver of metabolic stress, given its broad positive correlations with multiple unfavorable metabolites and negative correlations with protective metabolites. Although future validation is required, these multiomics analyses support the hypothesis that the persistent metabolic benefits of early pregnancy CDCA intervention are partially mediated by optimizing specific microbe–metabolite interactions, ultimately creating a superior maternal environment for fetal development.

In conclusion, this study demonstrates that early gestation CDCA supplementation acts as a potent nutritional programming strategy to significantly enhance reproductive performance. Our findings confirm that the anti-inflammatory and antioxidant benefits of CDCA persist into late gestation, effectively alleviating maternal metabolic stress. The sustained benefits are achieved through the targeted modulation of the gut microbiota, particularly by suppressing the potential pathogen *NK4A214_group* and the optimization of metabolic pathways related to lipid and amino acid metabolism, such as reducing LPCs and CML while enhancing L-ornithine and others. Collectively, this study proposes that CDCA can overcome the time limits of early pregnancy, producing systemic benefits throughout gestation, offering a practical approach to improve both maternal metabolic health and gestational outcomes.

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Author contributions

The authors' responsibilities were as follows – YZ: conceptualization, data curation, formal analysis, investigation, methodology and writing – original draft; XW: methodology and visualization; QM, EL, DZ, HL, LC, JW, and TF: investigation and methodology; MS: visualization and writing – review and editing; MC: conceptualization, data curation, formal analysis, investigation, funding acquisition, methodology, validation, visualization, resources, writing – original draft and review and editing; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Data availability

Data described in the manuscript will be made available upon request pending application and approval from the corresponding author.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Gemini in order to improve readability and language. After using this tool, the author reviewed and edited the content as needed and take full responsibility for the content of the publication.

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