This is a “preproof” accepted article for *Animal Nutriomics*.
This version may be subject to change during the production process.
10.1017/anr.2025.2

# Effects of early perinatal body condition in dairy cows on blood biochemical indices and liver metabolic mechanism in their offspring

**YanZhe Wang[1] YongJi Gao[1] Yu Zhang[2] ZhiYong Hu[1]***

**[1] College of Animal Science and Technology, Shandong Agricultural Universit,271001,Taian Shandong,SDAU**

**[2] College of Animal Science and Technology, Nanjing Agricultural University,210095,NanJing JiangSu,NJAU**

***Corresponding Author: ZhiYong Hu College of Animal Science and Technology, Shandong Agricultural Universit,271001,Taian Shandong,SDAU**

**E-mail : hzy20040111@126.com**



https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

**Abstract:**

The body condition of cows during the early perinatal period has a long-term impact on the health of their offspring calves; however, research on the mechanisms of liver metabolism in this context is limited. This study investigates the effects of pre-perinatal cow body condition score (BCS) on various blood biochemical, antioxidant, and immune indices in offspring calves. The calves were categorized into two groups based on their mothers' BCS: the high body condition group (OHBCS) and the low body condition group (OLBCS). The results indicate that the levels of INS, NEFA, BHB, IgG, GSH-Px, and SOD in the blood of the OHBCS group were significantly higher than those in the OLBCS group ($p < 0.05$). In contrast, serum levels of TNF-α and IL-2 in the OHBCS group were significantly lower than those in the OLBCS group ($p < 0.001$ and $p < 0.05$, respectively). Additionally, integrating metabolomic and transcriptomic data revealed that levels of the tricarboxylic acid (TCA) cycle, fatty acid β-oxidation, and ornithine cycling were reduced in the OHBCS group, whereas the ketogenic pathway and triglyceride synthesis pathway were enhanced. These findings elucidate the mechanisms by which pre-perinatal cow body condition score influences liver metabolism in offspring calves.

**Key words:** Body condition score; Perinatal period; Calf; Metabolomics; Transcriptomics; Blood biochemical index

**Introduction:**

The perinatal period is a crucial stage for fetal growth and development, significantly influencing the long-term growth, development, and functionality of fetal organs due to changes in maternal metabolic status (Brumby, 1960; Reynolds et al., 2022). Body condition score (BCS) serves as a key indicator of the metabolic status of dairy cows, reflecting their overall metabolic state (Roche et al., 2009; Vailati-Riboni et al., 2016). Maintaining an appropriate BCS in perinatal dairy cows is essential for the development and productivity of their offspring (Poczynek et al., 2023).

During the perinatal period, dairy cows undergo significant physiological changes, often resulting in a state of negative energy balance (NEB) due to higher energy demands than intake (Grummer, 1995). To manage NEB, cows mobilize substantial body fat reserves (Janovick et al., 2011). In

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

comparison to cows with a normal body condition score (BCS), obese cows exhibit greater fat mobilization and higher concentrations of non-esterified fatty acids (NEFA) in their blood (Akbar et al., 2015). Extensive fat mobilization is closely associated with reduced feed intake in obese cows, indicating a heightened metabolic challenge (Caldeira et al., 2007; Schuh et al., 2019). Furthermore, BCS during the perinatal period influences serum levels of zinc (Zn), manganese (Mn), and chromium (Cr), with lower concentrations of these trace elements observed in obese cows (Dehghan Shahreza et al., 2020). In cows with elevated prepartum BCS, serum levels of NEFA, beta-hydroxybutyrate (BHB), and glucose are significantly higher than those in cows with normal BCS (Schuh et al., 2019). The developing fetus is affected by maternal nutrition, and Barker's "developmental programming theory" posits that early maternal influences can lead to physiological and metabolic changes in the fetus, potentially impacting the offspring in both the short and long term (BARKER, 1998).

Current research on ruminants has primarily concentrated on the phenotypic effects of maternal influences on offspring, encompassing aspects such as growth rate, ketone body characteristics, milk production, and immune capacity (Alharthi et al., 2021; Lopes et al., 2021; López Valiente et al., 2021). However, investigations into the impact of maternal metabolic status during the perinatal period on the metabolic mechanisms of offspring remain limited. In both humans and mice, numerous epidemiological studies and experimental animal models have demonstrated that maternal metabolic disorders during pregnancy elevate the risk of metabolic diseases in offspring, resulting in long-term consequences (Jousse et al., 2011; Damm et al., 2016; Bendor et al., 2022). We hypothesize that varying body condition scores (BCSs) in perinatal dairy cows will influence the metabolism of their offspring.

The growth and development of calves prior to weaning are critical for their subsequent development and performance (Kertz et al., 2017). The metabolic capacity of calves before weaning is a significant factor influencing their growth, with the liver serving as the central organ for metabolism. This study examined the impact of varying body condition scores (BCS) in perinatal dairy cows on the metabolic status of their offspring by comparing differences in hepatic metabolites, gene expression, and blood biochemical indicators in the calves.

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

## Materials and Methods:

### 2.1 Experimental Animals:

The experiment was conducted from July 2023 to September 2023 at a dairy farm in Shandong Province. Thirty cows exhibiting significant differences in body condition were selected to participate in this experiment 14 days prior to the expected delivery date. The classification of the dairy cows in this study was relative, with a body condition score of 3.56 ± 0.21 designated as the high body condition group (OHBCS) and a body condition score of 3.09 ± 0.20 classified as the low body condition group (OLBCS). Specific body condition score data can be found in Supplementary Table 1 All cows had similar parity (1.9 ± 0.5) and were in good health. Following natural calving, ten healthy male calves with comparable birth dates were selected from each group. The calves were divided into two groups based on their mothers' BCS: offspring of high-BCS cows (OHBCS) and offspring of low-BCS cows (OLBCS). Calves were required to receive qualified colostrum within one hour of birth, and all test calves were transferred to the calf island on the day they turned one day old. According to the farm's feeding management protocol, calves were fed regular milk （The milk composition data for normal milk are presented in Supplementary Table 2.）starting at two days of age, administered twice daily (at 7:00 and 15:00). From 2 to 15 days of age, each feeding consisted of 2.5 liters, while from 16 to 30 days of age, each feeding increased to 3.0 liters. The body weight, body height, and body length of the one-month-old calves are presented in Supplementary Table 3 .

### 2.2 Sample Collection:

#### 2.2.1 Blood Sample Collection and Measurement:

At one month of age, 10 mL of blood was collected from the jugular vein using a disposable syringe. The blood was collected in coagulation-promoting tubes, allowed to stand for 30 minutes, and then centrifuged for 5 minutes at 3,000 r/min. The supernatant was aliquoted into 1.5 mL centrifuge tubes and immediately stored at -20°C. Blood biochemical indices, including total protein (TP), albumin (ALB), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol (TC), were measured using an

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

automated biochemical analyzer (Beckman Coulter, USA). Levels of immunoglobulin G (IgG), interleukin-6 (IL-6), interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), bovine leptin (LEP), bovine adiponectin (ADP), bovine insulin, bovine resistin, non-esterified fatty acids (NEFA), and beta-hydroxybutyrate (BHB) were determined using enzyme-linked immunosorbent assay (ELISA). Malondialdehyde (MDA) levels were measured using an MDA assay kit, superoxide dismutase (SOD) levels with an SOD assay kit, glutathione peroxidase (GSH-Px) using a GSH-Px assay kit, and total antioxidant capacity (T-AOC) with a T-AOC assay kit.

**2.2.2 Liver Sample Collection:**

The calves were slaughtered via exsanguination through the jugular vein. Immediately following slaughter, the abdomen was opened, and the liver was extracted. The liver from each calf was divided into three cryogenic vials, which were rapidly placed in liquid nitrogen and subsequently stored at -80°C for further non-targeted metabolomic and transcriptomic analyses.

**2.2.3 Liver Tissue Untargeted Metabolomics:**

Untargeted metabolomic experiments were conducted on the OHBCS (n=6) and OLBCS (n=6). For each sample, 100 μL was mixed with 400 μL of pre-cooled 80% methanol for resuspension in an EP tube. The tube was incubated on ice for 5 minutes, followed by centrifugation at 15,000g and 4°C for 20 minutes. An appropriate volume of the supernatant was collected and diluted with mass spectrometry-grade water to reduce the methanol content to 53%. Centrifugation was performed as previously described. The final analysis was conducted using liquid chromatography-mass spectrometry (LC-MS) at Wuhan Metware Biotechnology Co., Ltd.

**2.2.4 Liver Transcriptomics:**

RNA was extracted from the liver tissues of both groups (n=3) using TRIzol reagent (Invitrogen). Library construction was performed by Wuhan Metware Biotechnology Co., Ltd. In brief, total RNA from the liver tissues was extracted using TRIzol reagent (TianGen, China), and its quality was assessed using a NanoDrop spectrophotometer (Thermo Scientific, U.S.A.). To generate sequencing libraries, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads, which were then utilized to synthesize the second strand of cDNA. Subsequently, DNA fragments were enriched through a PCR reaction, followed by purification and quantification

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

using the Bioanalyzer 2100 system (Agilent Technologies Inc., U.S.A.). Finally, the constructed sequencing library was sequenced using the NovaSeq 6000 platform (Illumina, U.S.A.).

**2.2.5 Real-Time PCR (RT-qPCR):**

The gene expression of OHBCS (n = 3) and OLBCS (n = 3), along with the corresponding transcriptome samples, was analyzed using the QuantiTect SYBR Green PCR kit (Qiagen) on CFX96 thermal cyclers (Bio-Rad, Hercules, CA). The primers employed for quantitative reverse transcription polymerase chain reaction (qRT-PCR) are listed in Table 1, with GAPDH serving as the internal control. Melt curve analysis was performed to confirm the specificity of the amplification reaction.

**2.2.6 Data analysis and visualization**

In the untargeted metabolomics analysis, raw data obtained from mass spectrometry were converted to mzXML format using ProteoWizard. Subsequently, peak extraction, alignment, and retention time correction were conducted using XCMS software. Peaks with a missing rate exceeding 50% in each group were filtered out, and missing values were imputed utilizing the KNN method. Peak areas were corrected using Support Vector Regression (SVR). Metabolites were identified by searching an in-house database, integrating public databases and prediction libraries, and employing the metDNA method. Metabolites with a P-value  1 were deemed significantly different, whereas those with a P-value > 0.1 and a VIP value > 1 were considered to have a trend of significant difference. KEGG enrichment pathways with a P-value less than 0.05 relevant to this study were characterized using the MetaboAnalyst platform (MetaboAnalyst 6.0; www.metaboanalyst.ca).

For transcriptomics, reads were mapped to the reference genome using STAR software (version 2.5.3a), and gene expression levels were estimated using featureCounts (Subread-1.5.1; Bioconductor). Gene expression was quantified as RPKM (Reads Per Kilobase million Mapped Reads). Differentially expressed genes (DEGs) were defined as those with Padj  1.2 or < 0.8. KEGG pathway enrichment analysis of DEGs was conducted using the OmicShare platform (p-value < 0.05) (www.omicshare.com/tools). Additionally, all statistical analyses were performed using GraphPad Prism 8 and SPSS 26 (IBM), with results presented as Mean ± SEM. Two-tailed,

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

unpaired t-tests were employed to calculate p-values (* indicates $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$).

## 3. Results:

### 3.1 Effect of Different Prepartum Body Conditions in Dairy Cows on Blood Metabolic Parameters in Offspring Calves:

OHBCS exhibited significantly higher insulin (INS) levels compared to OLBCS ($p<0.001$). Furthermore, the concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHB) were also significantly elevated in the OHBCS group ($p<0.05$) as shown in Table 2. However, variations in prepartum body condition among dairy cows did not significantly influence the concentrations of total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), total cholesterol (TC), triglycerides (TG), leptin (LEP), adiponectin (ADP), high-density lipoprotein cholesterol (HDL-C), or low-density lipoprotein cholesterol (LDL-C) in the blood of the offspring ($p>0.05$).

### 3.2 Effect of Different Prepartum Body Conditions in Dairy Cows on Blood Immune and Antioxidant Parameters in Offspring Calves:

The blood levels of TNF-α in the OH BCS cows were significantly lower ($p<0.001$) compared to those in the OLBCS cows. Additionally, IL-2 levels were significantly lower ($p<0.05$), while IgG levels were significantly higher ($p<0.05$) in the OH BCS group (Table 3). Serum levels of GSH-Px and SOD were also significantly higher ($p<0.001$) in the OH BCS group than in the OLBCS group. However, no significant differences were observed in IL-6, T-AOC, or MDA levels between the two groups ($p>0.05$).

### 3.3 Effect of Different Prepartum Body Conditions in Dairy Cows on Liver Metabolomics in Offspring Calves:

In the OPLS-DA model, the two groups exhibited a clear spatial separation (Figure 1 A). Based on the MS detection data, a total of 105 differential metabolites ($p < 0.05$) and 28 metabolites with differential trends ($0.05 < p < 0.1$) were identified. Among these, 54 metabolites were upregulated,

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

and 51 were downregulated. Metabolites such as Suillin, Oxybutynin, Biphenyl-2,3-diol, Palmitic amide, Oxethazaine, [12]-Gingerdione, 16-Hydroxyhexadecanoic acid, 2-Decaprenyl-6-methoxy-3-methyl-1,4-benzoquinone, (8)-Gingerol, Sterculic acid, Imatinib, Ramiprilat, 6-Methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol, L-Tryptophan, Erythrose, Stachyose, Eicosapentaenoic acid, Erythritol, and Geniposidic acid were significantly enriched in the livers of OHBCS calves ($p < 0.05$). Conversely, metabolites such as L-Homophenylalanine, Biocytin, 4,5-Chrysenedicarboxylate, L-Kynurenine, Malonic semialdehyde, 2,3-Diaminopropionic acid, Crotonic acid, N-Acetylvanilalanine, Aniline, 5-Hydroxyindoleacetic acid, (R)-2,3-Dihydroxy-isovalerate, Indole-3-glycerol phosphate, 3-Methylcrotonyl Glycine, Rasagiline, D-Kynurenine, Salmeterol, Ascorbic acid, Tetragastrin, N-Isovaleroylglycine, and Palmitoyl Serinol were significantly enriched in the livers of OLBCS calves ($p < 0.05$). The top 50 differential metabolites between the two groups were further subjected to cluster analysis (Figure 1B).

To gain insights into the biological processes associated with the differential metabolites between the OHBCS and OLBCS groups, KEGG enrichment analysis was conducted. As shown in Figure 2, significant differences were observed in pathways related to pyruvate, aspartate, and glutamate metabolism; phenylalanine, tyrosine, and tryptophan biosynthesis; the TCA cycle; tryptophan metabolism; phenylalanine metabolism; pyruvate metabolism; biotin metabolism; glycine, serine, and threonine metabolism; and d-amino acid metabolism ($p < 0.05$).

### 3.4 Effect of Different Prepartum Body Conditions in Dairy Cows on Liver Transcriptomics in Offspring Calves:

Prepartum body conditions in dairy cows can influence the liver transcriptomes of their offspring. We analyzed the liver transcriptomes of calves from different groups using transcriptomic techniques. Figure 1 (A) demonstrates a clear spatial separation between the OHBCS and OLBCS, indicating significant differences between the samples. Differential gene selection criteria was based on $|\log2FC| \geq 1$ and $FDR < 0.05$, identifying 7177 differentially expressed genes, with 3871 upregulated and 3306 downregulated (Figure 2 B).

To further elucidate the biological functions of these differentially expressed genes, we conducted KEGG database annotations and hypergeometric test-based enrichment analyses. Seventeen

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

modules exhibiting similar gene expression patterns were identified through weighted gene co-expression network analysis (WGCNA), with modules related to human diseases excluded (Figure 3 C). The majority of genes were found to be enriched in metabolic pathways, This suggests that the influence of various body conditions on the liver of offspring calves primarily affects metabolism, aligning with the objectives of our study.

Further analysis of the differential metabolic pathways in liver samples between the two groups revealed significant differences ($p < 0.05$) in several processes, including steroid hormone biosynthesis, bile secretion, pentose and glucuronate interconversions, drug metabolism via cytochrome P450, unsaturated fatty acid biosynthesis, porphyrin metabolism, protein processing in the endoplasmic reticulum, retinol metabolism, fatty acid metabolism, fatty acid elongation, niacin and nicotinamide metabolism, aldosterone synthesis and secretion, mineral absorption, sphingolipid metabolism, glycine, serine, and threonine metabolism, protein digestion and absorption, various forms of O-glycan biosynthesis, cortisol synthesis and secretion, and histidine metabolism (Figure 3D). Quantitative PCR verification of the genes of interest confirmed consistency with transcriptomic results, thereby demonstrating the reliability of the transcriptomic data (Figure 4).

**4. Discussion:**

Compared to OLBCS, OHBCS calves exhibited significantly higher levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) in the blood ($p < 0.05$), which aligns with the findings of Alharthi et al. (Alharthi et al., 2021). In ruminants, NEFA and BHB are essential for maintaining energy balance. Offspring of obese cows often demonstrate compensatory growth during the first two months after birth (Poczynek et al., 2023). The elevated levels of NEFA and BHB may provide an adequate energy supply for ruminants, potentially serving as a significant driving force behind the compensatory growth observed in calves from obese mothers (Long et al., 2011). The notable increase in blood BHB levels in calves with OHBCS may be associated with enhanced ketogenesis in the liver. However, elevated fat mobilization also increases the metabolic burden on the liver, with offspring of obese mammalian mothers being at a higher risk of developing fatty liver (Hagström et al., 2021).

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

Leptin regulates feeding and energy expenditure through the hypothalamus, thereby maintaining body weight balance (Perucatti et al., 2006). In goats, leptin is associated with maternal BCS, offspring BCS, and feed intake; notably, overweight ewes exhibit a deficiency in neonatal leptin (Long et al., 2011). Conversely, a recent study on dairy cows found no correlation between maternal BCS and offspring leptin levels (Brown et al., 2023). Our study similarly found no significant difference in leptin levels between the OHBCS and OLBCS groups. Although the total protein content of the OHBCS group was higher than that of the OLBCS group, this difference was not statistically significant. Offspring calves from cows with a high body condition during the peripartum period experience greater nutritional restrictions in utero (Bernabucci et al., 2005a). Evidence suggests that intrauterine nutritional restriction during the peripartum period can enhance the permeability of the small intestine to macromolecular proteins, facilitating greater absorption of proteins from milk and subsequently increasing total blood protein levels (Sangild, 2003). No significant differences in blood urea nitrogen levels were observed between the OHBCS and OLBCS groups; however, the elevated levels in the OLBCS group may indicate increased amino acid deamination activity, which provides a carbon skeleton for gluconeogenesis (Hammon et al., 2013).

Glutathione peroxidase directly interacts with free radicals and lipid peroxides, thereby safeguarding the body from oxidative damage. Consequently, glutathione serves as a vital indicator of antioxidant activity (Forman et al., 2009). Similarly, superoxide dismutase functions as an antioxidant enzyme that constitutes the primary intracellular antioxidant pathway. In this study, the levels of glutathione peroxidase and superoxide dismutase in the OHBCS group were significantly higher than those in the OLBCS group ($p < 0.001$). This difference may be attributed to the elevated levels of NEFA and BHB in the bloodstream of the OHBCS group ($p < 0.05$). Elevated concentrations of glutathione peroxidase and superoxide dismutase are essential for protecting the body from oxidative damage and for maintaining a normal physiological state. Previous studies have demonstrated that plasma glutathione peroxidase levels correlate with plasma lipid levels (Tüzün et al., 2002), and that increased concentrations of NEFA and BHB in dairy cows are associated with heightened oxidative stress (Bernabucci et al., 2005b).

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

Immunoglobulin G (IgG) is the predominant immunoglobulin in the body, facilitating the phagocytosis of pathogens by immune cells and neutralizing bacterial toxins (Shi et al., 2020). Elevated IgG levels can enhance immune function in dairy cows (Xu et al., 2017). The concentration of IgG in the OHBCS serum was significantly higher than that in the OLBCS serum, possibly due to the greater nutritional restrictions experienced by the maternal OHBCS during the peripartum period. Recent studies indicate that offspring of nutritionally restricted cows exhibit higher passive immunity coefficients for IgG (Wichman et al., 2023). A limitation of this study is the absence of measurements for other immunoglobulins, such as immunoglobulin A and immunoglobulin M.

The liver serves as a central hub for metabolic pathways in the body. To further investigate the mechanisms by which various body conditions in cows during the peripartum period affect their offspring, we employed untargeted metabolomics and transcriptomics to assess liver metabolites and differential gene expression, with a particular emphasis on liver-involved metabolic pathways. Succinate, α-ketoglutaric acid, and malate are critical intermediates of the tricarboxylic acid (TCA) cycle. Notably, pyruvate, succinate, and malate levels were lower in the OHBCS group compared to the OLBCS group. Furthermore, the genes IDH1 and SUCLA2, which are part of the TCA pathway, exhibited significant inhibition in the OHBCS group ($p < 0.001$). Additionally, pyruvate an essential substrate of the TCA pathway, was also significantly decreased in the OHBCS group. The genes encoding the pyruvate dehydrogenase complex, such as PDHA1 and DLD , were significantly downregulated in the OHBCS group, indicating that the conversion of pyruvate to acetyl-CoA was inhibited in this group. These findings suggest that the TCA pathway was inhibited in the OHBCS group (as illustrated in Figure 5). Glycolysis is the primary pathway for pyruvate production, which is subsequently linked to other biochemical pathways. In this study, two key genes in the glycolysis pathway, G6PC1 and PDHA1, were significantly inhibited, indicating a marked downregulation of the glycolysis pathway in the OHBCS group. This downregulation may represent another significant factor contributing to the decreased levels of pyruvate.

Through KEGG enrichment analysis of the transcriptome and metabolome, we identified significant differences in metabolic pathways related to lipid metabolism. However, there were no

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

differences in the gene expression of CD36, FABP3, LPL, and SLC27A1, which are involved in fatty acid transport. Notably, ACSL1 and CPT2 were significantly upregulated in OHBCS($p < 0.0001$). ACSL1 encodes enzymes from the long-chain acyl-CoA synthetase family that catalyze the initial step of intracellular fatty acid activation, while CPT2 plays a crucial role in the fatty acid oxidation pathway, indicating a reduced capacity for fatty acid β-oxidation. Furthermore, DGAT1, which is involved in triglyceride structural formation by catalyzing the acylation of glycerol with fatty acids (Ali et al., 2021), was significantly upregulated in OHBCS compared to OLBCS ($p < 0.0001$). This suggests that the triglyceride synthesis pathway is upregulated in the OHBCS group compared to the OLBCS group, potentially leading to increased lipid accumulation in the liver of OHBCS. Evidence indicates that lipid accumulation is associated with TCA cycle capacity (Koves et al., 2008). Additionally, DPP4 was significantly downregulated in OHBCS ($p < 0.001$). DPP4 plays a crucial role in liver glucose metabolism and homeostasis, affecting insulin sensitivity and hepatic fat deposition (Miyazaki et al., 2012; Aroor et al., 2015; Baumeier et al., 2017). In calves subjected to peripartum nutritional restriction, the expression of DPP4 is also limited (Muroya et al., 2022). The expression of angiopoietin-like protein 8 (ANGPTL8) is closely related to glucose and lipid metabolism (Ye et al., 2023). Although the role of ANGPTL8 in glucose and lipid metabolism remains controversial, its upregulation in the liver may be associated with disorders of glucose and lipid metabolism (Fenzl et al., 2014; Hu et al., 2014; Zhang et al., 2020). In our study, ANGPTL8 expression was significantly elevated in offspring of cows with high body condition scores OHBCS ($p < 0.001$), indicating a heightened risk of hepatic glucose and lipid metabolism disorders in these calves during the peripartum period. However, further long-term follow-up studies are warranted. Additionally, ACACA, the rate-limiting enzyme in de novo fatty acid synthesis, converts acetyl-CoA to fatty acids and plays a crucial role in this process (Wang et al., 2022). In OHBCS, ACACA expression was significantly decreased ($p < 0.001$), suggesting a diminished hepatic capacity for fatty acid synthesis, which is accompanied by reduced acetyl-CoA flux into the fatty acid synthesis pathway. This reduction implies that acetyl-CoA is more involved in the ketogenic pathway, as illustrated in Figure 5. A study examining the hepatic metabolic mechanisms in dairy cows during the perinatal period also found that acetyl-CoA generated from fatty acid β-oxidation is utilized in ketogenesis (Ghaffari et al., 2021). This

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

finding explains why blood BHB levels were significantly higher in the OHBCS group compared to the OLBCS group.

The metabolism of amino acids in offspring is influenced by maternal nutritional status during pregnancy (Gruppuso et al., 2005). Research on epigenetic modifications has demonstrated that maternal nutrition during pregnancy affects the promoter methylation levels of enzymes involved in the urea cycle and tyrosine aminotransferase in the liver of offspring (Muroya et al., 2023). In this study, KEGG enrichment analysis of differential metabolites revealed significant differences in multiple amino acid metabolism pathways between OHBCS and OLBCS (Figure 4-3). Metabolomic KEGG enrichment analysis indicated significant reductions in the glycine, cysteine, and serine metabolic pathways in OHBCS ($p < 0.05$). These amino acids are all glycogenic; therefore, the reduction in their metabolic levels also diminishes the supply of raw materials for the glucose metabolic pathway to some extent, which correspondingly reduces the glucose metabolism level, including TCA, in the OHBCS group. In the phenylalanine, tyrosine, and tryptophan synthesis pathways, phenylalanine and tyrosine levels were significantly higher in OHBCS than in OLBCS. Studies have indicated that tyrosine is elevated in the offspring of obese and type 2 diabetes patients compared to those of normal offspring (Villarreal-Pérez et al., 2014). Phenylalanine and tyrosine function as both ketogenic and glucogenic amino acids, generating acetoacetic acid in the ketogenic pathway and fumarate in the glucogenic pathway. Although acetoacetic acid and fumarate were not directly detected in the metabolomic data, we speculate that the reduced requirement for fumarate in the TCA cycle in OHBCS, combined with the significant elevation of β-hydroxybutyrate in the blood, suggests that phenylalanine, tyrosine, and tryptophan primarily contribute to the ketogenic pathway. Furthermore, amino acid metabolism generates substantial amounts of ammonia, which is converted to urea and excreted via the ornithine cycle. The ornithine cycle is an energy-consuming process; consequently, the levels of the TCA cycle and the capacity for fatty acid β-oxidation in the OHBCS group are lower than those in the OLBCS group. This reduction results in the liver lacking sufficient energy for the urea cycle. Additionally, the decreased levels of CSP-1 ($p< 0.001$) in the arginine cycle pathway indicate a diminished urea cycle pathway in the OHBCS group. Furthermore, the blood urea nitrogen content in the OHBCS group was lower than that in the OLBCS group. Based on this

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

evidence, we speculate that the OHBCS group experiences a greater cumulative ammonia pressure.

**Conclusion:**

This study selected two groups of cows that exhibited a significant difference in BCS during the pre-perinatal period, aiming to examine the impact of BCS on the metabolic mechanisms of offspring calves. It was observed that liver glycolysis and TCA cycle levels, along with fatty acid β-oxidation and arginine cycle were inhibited, while triglyceride synthesis and the ketogenic pathway were increased. Consequently, the concentrations of NEFA and BHB in systemic circulation were significantly elevated, suggesting that the liver's energy supply in the OHBCS group was lower than that in the OLBCS group. Additionally, the OHBCS group required more ketone bodies to meet energy demands, which may lead to adverse effects associated with elevated ketone bodies and liver triglyceride deposition; these negative effects warrant long-term investigation. Furthermore, we found that the antioxidant stress capacity of offspring calves with high body condition during the perinatal period was superior to that of calves with a low body condition. The next phase of this study will focus on the metabolism of muscle and adipose tissue in calves.

**Acknowledgements：**

The authors would like to express their gratitude to the staff of Shandong Gaosu Bioengineering Co., Ltd. (Jinan, China) for their assistance with animal husbandry and sampling.

**Funding：**

This research was supported by grants from the National Natural Science Foundation of China (Grant Number: 32172760) and was also funded by the Modern Agro-industry Technology Research System of China (Grant Number: CARS-36).

**Contributions：**

ZH and YW designed the study; YZ, YW and YG conducted the trial; YG analyzed the blood sample data, YW analyzed the liver sample data, YW completed the writing of the paper, ZH provided the project funding and revised the manuscript, and all authors read and approved the final manuscript.

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

**Ethics declarations：**

Competing interests：

The authors declare no competing interests.

**Reference:**

Akbar. H., T.M. Grala, M. Vailati Riboni,. et al (2015) Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows. JOURNAL OF DAIRY SCIENCE 98:1019–1032.

Alharthi, A.S., D.N. Coleman, I.A. Alhidary. et al (2021) Maternal body condition during late-pregnancy is associated with in utero development and neonatal growth of Holstein calves. JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY . Apr 2;12(1):44

Ali. I., C. Li, L. Li. et al (2021) Effect of acetate, β-hydroxybutyrate and their interaction on lipogenic gene expression, triglyceride contents and lipid droplet formation in dairy cow mammary epithelial cells. IN VITRO CELL.DEV.BIOL.-ANIMAL 57:66–75.

Aroor, A.R., J. Habibi. et al (2015) Dipeptidyl Peptidase-4 Inhibition Ameliorates Western Diet–Induced Hepatic Steatosis and Insulin Resistance Through Hepatic Lipid Remodeling and Modulation of Hepatic Mitochondrial Function. DIABETES 64:1988–2001.

BARKER, D.J.P. (1998) In utero programming of chronic disease. CLINICAL SCIENCE 95:115–128.

Baumeier. C., L. Schlüter. S. Saussenthaler. et al (2017) Elevated hepatic DPP4 activity promotes insulin resistance and non-alcoholic fatty liver disease. MOL METAB 6:1254–1263.

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

Bendor. C.D., A. Bardugo, R.S. Rotem. et al (2022) Glucose Intolerance in Pregnancy and Offspring Obesity in Late Adolescence. DIABETES CARE 45:1540–1548.

Bernabucci. U., B. Ronchi., N. Lacetera. et al (2005a) Influence of Body Condition Score on Relationships Between Metabolic Status and Oxidative Stress in Periparturient Dairy Cows. JOURNAL OF DAIRY SCIENCE 88:2017–2026.

Bernabucci. U., B. Ronchi., N. Lacetera. et al (2005b) Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. J DAIRY SCI 88:2017–2026.

Brown. W.E., H.T. Holdorf., S.J. Kendall. et al (2023) Dam Body Condition Score Alters Offspring Circulating Cortisol and Energy Metabolites in Holstein Calves but Did Not Affect Neonatal Leptin Surge. METABOLITES 13:631.

Brumby, P.J. (1960) The influence of the maternal environment on growth in mice. HEREDITY 14:1–18.

Caldeira. R.M., A.T. Belo. et al (2007) The effect of body condition score on blood metabolites and hormonal profiles in ewes. SMALL RUMINANT RESEARCH 68:233–241.

Damm. P., A. Houshmand-Oeregaard, L. Kelstru. et al (2016) Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. DIABETOLOGIA 59:1396–1399.

Dehghan Shahreza. F., H.A. Seifi, and M. Mohri. (2020) The effects of dry period body condition

score on some trace minerals, oxidative, and inflammatory indicators in transition dairy cows. ANIM. PROD. SCI. 60:363.

Fenzl. A., B.K. Itariu, L. Kosi. et al (2014) Circulating betatrophin correlates with atherogenic lipid profiles but not with glucose and insulin levels in insulin-resistant individuals. DIABETOLOGIA 57:1204–1208.

Forman. H.J., H. Zhang, and A. Rinna. (2009) Glutathione: Overview of its protective roles, measurement, and biosynthesis. MOLECULAR ASPECTS OF MEDICINE 30:1–12.

Ghaffari. M.H., M.T. Alaedin, H. Sadri. et al (2021) Longitudinal changes in fatty acid metabolism and in the mitochondrial protein import system in overconditioned and normal conditioned cows: A transcriptional study using microfluidic quantitative PCR. JOURNAL OF DAIRY SCIENCE 104:10338–10354.

Gómez-Vilarrubla. A., B. Mas-Parés, G. Carreras-Badosa. et al (2023) Placental epigenetic marks related to gestational weight gain reveal potential genes associated with offspring obesity parameters. OBESITY 31:1903–1912.

Grummer, R.R. (1995) Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. JOURNAL OF ANIMAL SCIENCE 73:2820.

Gruppuso.P.A., J.M. Boylan, P. Anand. et al (2005) Effects of Maternal Starvation on Hepatocyte Proliferation in the Late Gestation Fetal Rat. PEDIATR RES 57:185–191.

Hagström. H., T.G. Simon, B. Roelstraete. et al (2021) Maternal obesity increases the risk and

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

severity of NAFLD in offspring. JOURNAL OF HEPATOLOGY 75:1042–1048.

Hammon. H.M., J. Steinhoff-Wagner, J. Flor. et al (2013) Lactation Biology Symposium: role of colostrum and colostrum components on glucose metabolism in neonatal calves. J ANIM SCI 91:685–695.

Hu. H., W. Sun, S. Yu. et al (2014) Increased circulating levels of betatrophin in newly diagnosed type 2 diabetic patients. DIABETES CARE 37:2718–2722.

Janovick. N.A., Y.R. Boisclair, and J.K. Drackley. (2011) Prepartum dietary energy intake affects metabolism and health during the periparturient period in primiparous and multiparous Holstein cows. JOURNAL OF DAIRY SCIENCE 94:1385–1400.

Jousse. C., L. Parry, S. Lambert-Langlais. et al (2011) Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: implication for the understanding of metabolic syndrome. FASEB J 25:3271–3278.

Kertz. A.F., T.M. Hill, J.D. Quigley. et al (2017) A 100-Year Review: Calf nutrition and management. JOURNAL OF DAIRY SCIENCE 100:10151–10172.

Koves. T.R., J.R. Ussher, R.C. Nolan. et al (2008) Mitochondrial Overload and Incomplete Fatty Acid Oxidation Contribute to Skeletal Muscle Insulin Resistance. CELL METABOLISM 7:45–56.

Long. N.M., S.P. Ford, and P.W. Nathanielsz. (2011) Maternal obesity eliminates the neonatal lamb plasma leptin peak. THE JOURNAL OF PHYSIOLOGY 589:1455–1462.

Lopes. M.G., A.S. Alharthi, V. Lopreiato. (2021) Maternal body condition influences neonatal calf whole-blood innate immune molecular responses to ex vivo lipopolysaccharide challenge. JOURNAL OF DAIRY SCIENCE 104:2266–2279.

López Valiente. S., A.M. Rodriguez, N.M. Long. et al (2021) The degree of maternal nutrient restriction during late gestation influences the growth and endocrine profiles of offspring from beef cows. ANIM. PROD. SCI. 62:163–172.

Miyazaki. M., M. Kato, K. Tanak. et al (2012) Increased hepatic expression of dipeptidyl peptidase-4 in non-alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. MOLECULAR MEDICINE REPORTS 5:729–733.

Muroya. S., K. Otomaru, K. Oshima. et al (2023) DNA Methylation of Genes Participating in Hepatic Metabolisms and Function in Fetal Calf Liver Is Altered by Maternal Undernutrition during Gestation. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 26;24(13):10682.

Muroya. S., Y. Zhang, K. Otomaru. et al (2022) Maternal Nutrient Restriction Disrupts Gene Expression and Metabolites Associated with Urea Cycle, Steroid Synthesis, Glucose Homeostasis, and Glucuronidation in Fetal Calf Liver. METABOLITES 12:203.

Parkes. H.A., E. Preston, D. Wilks. et al (2006) Overexpression of acyl-CoA synthetase-1 increases lipid deposition in hepatic (HepG2) cells and rodent liver in vivo. AMERICAN JOURNAL OF PHYSIOLOGY-ENDOCRINOLOGY AND METABOLISM 291:E737–E744.

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

Perucatti. A., G.P. Di Meo, M. Vallinoto. et al (2006) FISH-mapping of LEP and SLC26A2 genes in sheep, goat and cattle R-banded chromosomes: comparison between bovine, ovine and caprine chromosome 4 (BTA4/OAR4/CHI4) and human chromosome 7 (HSA7). CYTOGENETIC AND GENOME RESEARCH 115:7–9.

Poczynek. M., L. de S. Nogueira, I.F. Carrari. et al (2023) Associations of Body Condition Score at Calving, Parity, and Calving Season on the Performance of Dairy Cows and Their Offspring. ANIMALS 13:596.

Reynolds. L.P., W.J.S. Diniz, M.S. Crouse. et al (2022) Maternal nutrition and developmental programming of offspring. REPROD FERTIL DEV 35:19–26.

Roche. J.R., N.C. Friggens, J.K. Kay. et al (2009) Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J DAIRY SCI 92:5769–5801.

Sangild, P.T. (2003) Uptake of Colostral Immunoglobulins by the Compromised Newborn Farm Animal. ACTA VETERINARIA SCANDINAVICA 44:S105.

Schuh. K., H. Sadri, S. Häussler. et al (2019) Comparison of performance and metabolism from late pregnancy to early lactation in dairy cows with elevated v. normal body condition at dry-off. ANIMAL 13:1478–1488.

Shi. L., Y. Xu, C. Mao. et al (2020) Effects of heat stress on antioxidant status and immune function and expression of related genes in lambs. INT J BIOMETEOROL 64:2093–2104.

Tüzün. A., A. Erdil, V. İnal. et al (2002) Oxidative stress and antioxidant capacity in patients with

inflammatory bowel disease. CLINICAL BIOCHEMISTRY 35:569–572.

Vailati-Riboni. M., S. Meier, C.R. Burke. et al (2016) Prepartum body condition score and plane of nutrition affect the hepatic transcriptome during the transition period in grazing dairy cows. BMC GENOMICS 17:854

Villarreal-Pérez, J.Z., J.Z. Villarreal-Martínez. et al (2014) Plasma and urine metabolic profiles are reflective of altered beta-oxidation in non-diabetic obese subjects and patients with type 2 diabetes mellitus. DIABETOLOGY & METABOLIC SYNDROME 6:129.

Wang. S., F. Hu, Q. Diao. et al (2022) Comparison of Growth Performance, Immunity, Antioxidant Capacity, and Liver Transcriptome of Calves between Whole Milk and Plant Protein-Based Milk Replacer under the Same Energy and Protein Levels. ANTIOXIDANTS 11:270.

Wichman, L.G., C.A. Redifer, and A.M. Meyer. (2023) Maternal nutrient restriction during late gestation reduces vigor and alters blood chemistry and hematology in neonatal beef calves. JOURNAL OF ANIMAL SCIENCE 101:skad342.

Xu H., W. Huang, Q. Hou. et al (2017) The effects of probiotics administration on the milk production, milk components and fecal bacteria microbiota of dairy cows. SCI BULL (BEIJING) 62:767–774.

Ye. H., Q. Zong, H. Zou. et al (2023) Emerging insights into the roles of ANGPTL8 beyond glucose and lipid metabolism. FRONT. PHYSIOL 1:14:1275485.

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

Zhang. Z., H. Wu, L. Dai. et al (2020) ANGPTL8 enhances insulin sensitivity by directly activating insulin-mediated AKT phosphorylation. GENE 749:144707.

**Table 1.RT-qPCR gene sequence**

| | |
|---|---|
| G6PC1 | AAAAGCCAACCTACAGATTTCG<br>TGAGCAGCAAGGTAGATTCG |
| CPT2 | CACCATTAGAAGATACCTCAGTGC<br>TCCAGTTTCAAAACTCTTACACAACT |
| ACSL1 | ACTGCTCCACAAGGGCTTC<br>CATCCCTGTTCAATGATCACC |
| ASL | TCAGAACCATGGTGGATCG<br>CCTCTGCAGGTGTGTGTACC |
| β-actin | AACTCCATCATGAAGTGTGACG<br>GATCCACATCTGCTGGAAGG |

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

**Table2. Effects of different body conditions of cows in early perinatal period on blood metabolic parameters of offspring calves**

| Items | Groups | | SEM | *P*-value |
|---|---|---|---|---|
| | OHBCS | OLBCS | | |
| TP(g/L) | 89.89±22.93 [a] | 77.53±14.32 [a] | 6.37 | 0.189 |
| ALB(g/L) | 26.69±6.47 [a] | 22.43±3.56 [a] | 1.74 | 0.103 |
| ALT (U/L) | 16.14±4.81 [a] | 15.53±2.67 [a] | 1.30 | 0.743 |
| AST (U/L) | 72.02±18.23 [a] | 76.82±13.11 [a] | 5.29 | 0.530 |
| BUN | 4.97±2.22 [a] | 5.67±3.90 [a] | 1.06 | 0.645 |
| TG(mmol/L) | 1.89±1.72 [a] | 1.83±0.13 [a] | 0.05 | 0.432 |
| TC(mmol/L) | 0.63±0.17 [a] | 0.61±0.16 [a] | 0.06 | 0.790 |
| LEP (ug/L) | 22.83±5.40 [a] | 26.87±4.36 [a] | 1.64 | 0.101 |
| ADP（ng/ml) | 22.87±5.49 [a] | 27.17±5.75 [a] | 1.87 | 0.125 |
| INS（mIu/L） | 16.88±0.98 [a] | 15.06±1.23 [b] | 0.37 | <0.001 |
| NEFA(umol/L) | 265.94±31.39 [a] | 230.29±27.48[b] | 9.83 | 0.021 |
| BHBA（ng/mL） | 332.73±22.18[a] | 284.30±55.12 [b] | 14.00 | 0.026 |
| HDL-C(mmol/L) | 1.16±0.07 [a] | 1.15±0.10 [a] | 0.03 | 0.670 |
| LDL-C(mmol/L) | 0.50±0.09 [a] | 0.41±0.10 [a] | 0.03 | 0.060 |

**TP: Total protein ALB: Albumin ALT: Alanine aminotransferase; AST: Aspartate transaminase; GLU: Glucose; BUN: Blood urea nitrogen;TG: Triglycerides; TC: Total cholesterol; LEP: Leptin; ADP: Adiponectin; INS: Insulin; NEFA: Non-esterified fatty acid; BHBA: Beta-hydroxybutyrate; HDL-C: High density lipoprotein; LDL-C: Low density lipoprotein. Lowercase letters with the same data superscript in the table indicate no significant difference (P > 0.05), while different lowercase letters indicate significant difference (P < 0.05).**

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

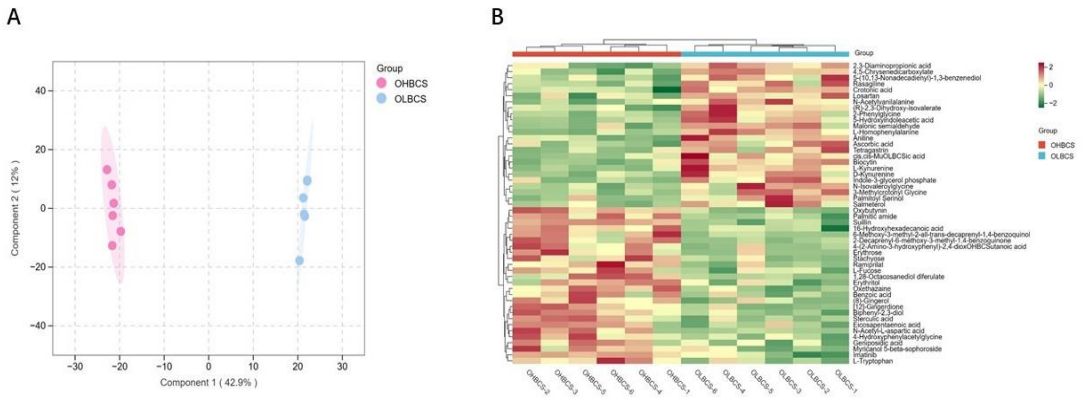
**Table 3 Effects of different body conditions of cows in the early perinatal period on blood immunity and antioxidant indexes of their offspring calves**

| Items | Groups | | SEM | *P*-value |
|---|---|---|---|---|
| | OHBCS | OLBCS | | |
| IgG(mg/mL) | 45.32±6.76 [a] | 38.73±6.34 [b] | 2.19 | 0.049 |
| TNF-α(pg/L) | 137.58±16.44 [a] | 166.68±18.18 [b] | 5.78 | <0.001 |
| IL-2(pg/mL) | 1274.43±270.27 [a] | 1505.72±171.42 [b] | 75.44 | 0.046 |
| IL-6(pg/mL) | 499.11±85.25 [a] | 555.38±62.84 [a] | 24.96 | 0.131 |
| T-AOC(U/mL) | 26.70±3.18 [a] | 23.09±5.01 [a] | 1.40 | 0.0868 |
| GSH-Px(nmol/ml) | 271.14±28.26 [a] | 227.20±29.86 [b] | 9.69 | <0.001 |
| MDA(nmol/mL) | 1.65±0.46 [a] | 1.90±0.47 [a] | 0.15 | 0.279 |
| SOD(U/mL) | 80.22±9.20 [a] | 57.48±5.09 [b] | 2.48 | <0.001 |

**IgG: Immunoglobulin G; TNF-α: Tumor necrosis factor-α; IL-2: Interleukin -2; IL-6: Interleukin -6; T-AOC: Total antioxidant capacity; GSH-Px: Glutathione peroxidase; MDA: Malondialdehyde; SOD: Superoxide dismutase. Lowercase letters with the same data superscript in the table indicate no significant difference (P > 0.05), while different lowercase letters indicate significant difference (P < 0.05).**

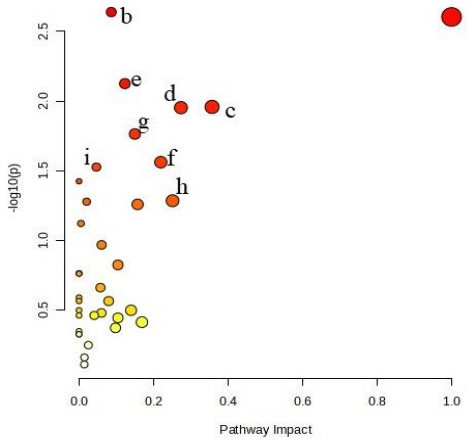
https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

**Figure 1. Analysis of different metabolites between perinatal high body condition dairy cow progeny (OHBCS) and perinatal low body condition dairy cow progeny (OLBCS)**




**（A）score map of liver metabolite OPLS-DA. （B）expression profiles of the top 50 differential metabolites.**

**Figure 2. KEGG enrichment analysis of different differential metabolites.**




a

a: Phenylalanine, tyrosine and tryptophan biosynthesis
b: Alanine, aspartate and glutamate metabolismc:
c: Phenylalanine metabolism
d: Pyruvate metabolism
e: Citrate cycle (TCA cycle)
f: Glyoxylate and dicarboxylate metabolism
g: Biotin metabolism
h: D-Amino acid metabolism
i: Glycine, serine and threonine metabolism

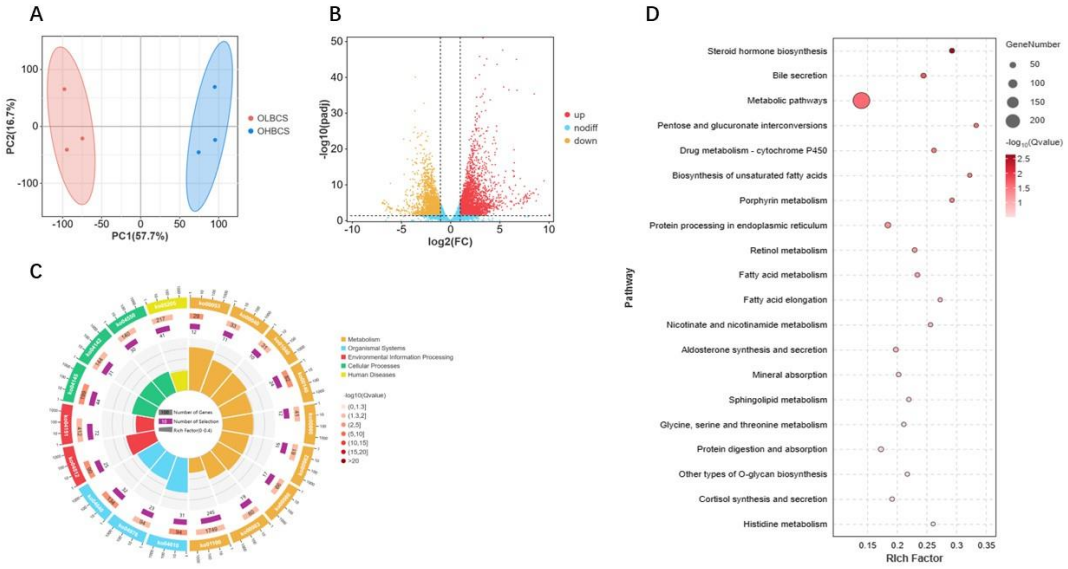
https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

| Metabolism/Pathway | Hits/Total Metabolites | p-Value | Increased in OHBCS | Decreased in OHBCS |
|---|---|---|---|---|
| Alanine, aspartate and glutamate metabolism | 4/28 | 0.0022964 | N-Acetyl-L-aspartate、N-Carbamoyl-L-aspartate | (S)-Malate、Pyruvate |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 2/4 | 0.0024891 | L-Phenylalanine、L-Tyrosine | |
| Citrate cycle (TCA cycle) | 3/20 | 0.0074972 | | Succinate、(S)-Malate、Pyruvate |
| Phenylalanine metabolism | 2/8 | 0.01102 | L-Phenylalanine、L-Tyrosine | |
| Pyruvate metabolism | 3/23 | 0.011158 | | (R)-S-Lactoylglutathione、Pyruvate、(S)-Malate |
| Biotin metabolism | 2/10 | 0.017252 | L-Lysine、 | |
| Glyoxylate and dicarboxylate metabolism | 3/32 | 0.027493 | Hydroxypyruvate | (S)-Malate、Pyruvate |
| Glycine, serine and threonine metabolism | 3/33 | 0.029816 | Hydroxypyruvate | D-Serine、Pyruvate |
| D-Amino acid metabolism | 2/15 | 0.037709 | D-Proline | D-Serine |

**The progeny of perinatal cows with high body condition were represented by OHBCS, and those of perinatal cows with low body condition were represented by OLBCS**

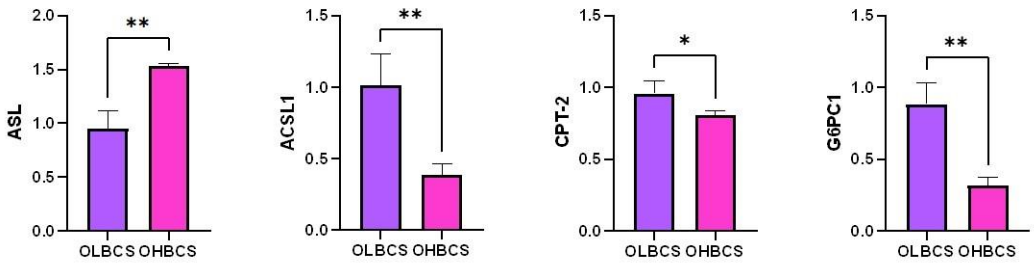
https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

**Figure 3.Transcriptome differential analysis of perinatal high body condition dairy cow progeny (OHBCS) and perinatal low body condition dairy cow progeny (OLBCS)**



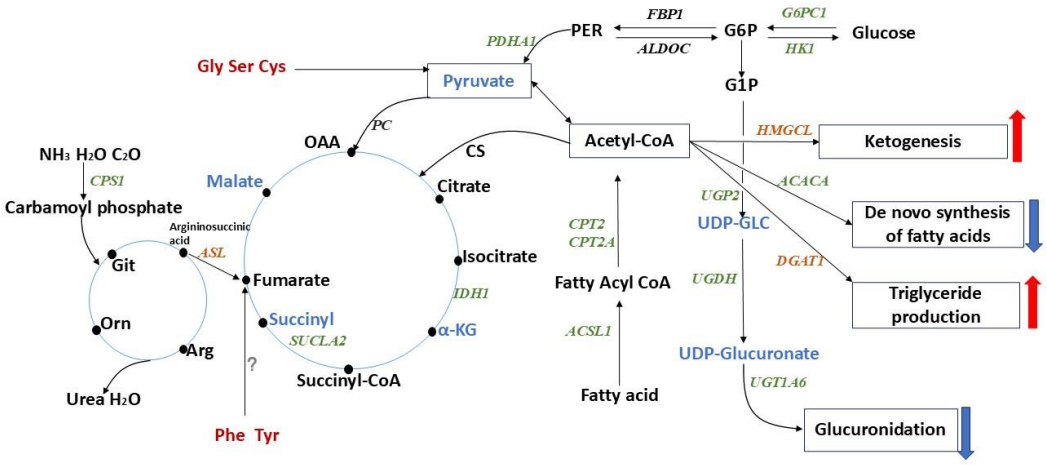

**（A） PCA of liver transcriptome. （B） transcriptome differential gene volcano map. （C） Gene co-expression network analysis. KO (KEGG Ontology) enrichment circle diagram of cyan module (from the outside to the inside, the first circle represents enrichment pathways, and the number outside the circle is the coordinate ruler of the number of genes; The second circle represents the number and Q value of background genes in this pathway, and the more genes, the longer the bar; The third circle represents the number of the DEGs in this pathway; The fourth circle represents the value of Rich Factor in each pathway) Gene co-expression network analysis. KO (KEGG Ontology) enrichment circle diagram of cyan module (from the outside to the inside, the first circle represents enrichment pathways, and the number outside the circle is the coordinate ruler of the number of genes; The second circle represents the number and Q value of background genes in this pathway, and the more genes, the longer the bar; The third circle represents the number of the DEGs in this pathway; The fourth circle represents the value of Rich Factor in each pathway) （D）KEGG enrichment of differential genes in OLBCS and OHBCS groups (showing only the top 20 most significant pathways in Metabolism)**

**Figure 4. q-pcr verification results of differential genes in OLBCS and OHBCS groups**




https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press

**Figure 5. Hypothesized mechanisms of different body conditions of perinatal cows on liver metabolism of offspring calves.**




**Genes rather than metabolites are shown in italics, green and blue for declines in the OHBCS group, and red and orange-yellow for increases**

https://doi.org/10.1017/anr.2025.2 Published online by Cambridge University Press