Microbiome

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Maternal gastrointestinal microbiome shapes gut microbial function and resistome of newborns in a cow-to-calf model

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Abstract

Background The maternal gut microbiome is the direct and important source of early colonization and development of the neonatal gut microbiome. However, differences in unique and shared features between mothers with different physiological phenotypes and their newborns still lack exhaustive investigation. Here, using a cow-to-calf model, a comprehensive investigation was conducted to elucidate the pattern and characterization of microbial transfer from the maternal source to the offspring.

Results The microbiota in the rumen and feces of dairy cows were divided into two clusters via enterotype analysis. The cows from the enterotype distinguished by *Prevotella* in the rumen had better production performance, whereas no difference was observed in the cows classified by feces enterotype. Furthermore, through a pairwise combination of fecal and ruminal enterotypes, we screened a group of dairy cows with excellent phenotypes. The gastrointestinal microbiomes of cows with different phenotypes and their offspring differed significantly. The rumen was a more important microbial source for meconium than feces. Transmission of beneficial bacteria from mother to offspring was observed. Additionally, the meconium inherits advantageous metabolic functions of the rumen. The resistome features of the rumen, feces, and meconium were consistent, and resistome abundance from cows to calves showed an expanding trend. The interaction between antibiotic-resistance genes and mobile genetic elements from the rumen to meconium was the most remarkable. The diversity of core metabolites from cows to calves was stable and not affected by differences in phenotypes. However, the abundance of specific metabolites varied greatly.

Conclusions Our study demonstrates the microbial taxa, metabolic function, and resistome characteristics of maternal and neonatal microbiomes, and reveals the potential vertical transmission of the microbiome from a cow-to-calf model. These findings provide new insights into the transgenerational transmission pattern of the microbiome.

Keywords Enterotype, Microbiome, Multi-omics, Vertical transmission, Resistome

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Introduction

The gastrointestinal (GIT) microbiota is considered an important "microbial organ" in human and animal hosts [1]. The gut microbiome plays a critical role in host development [2], immune regulation [3], nutrition, and metabolism and can be influenced or regulated by diet, environment, disease, and antibiotic treatment. Breaking the interaction balance has been reported to cause the stress response of the host and even induce diseases [4] including not only gut diseases such as inflammatory bowel diseases (IBDs), colorectal cancer, and Crohn's disease but also type 2 diabetes, asthma, and depression [5]. Especially during pregnancy, the sensitive gut microbiome is more susceptible to disturbance, and importantly, the changed microbiome not only directly affects the mother but also has a potential negative impact on their offspring [6, 7]. The maternal microbiome is the most direct and important source for the early colonization and development of the neonatal gut microbiome [8] and gut microbiota evolution in the early stage of the host determines its lifelong consequences [9, 10]. Many studies have described the profile of early-life microbiota and its potential relationship with maternal characteristics. However, the differences in unique and shared features between mothers with different physiological phenotypes and their newborns, from microbial structure and signature taxa to metabolic functions and resistome, have not been exhaustively investigated.

Dairy cattle (*Bos taurus*), a domestic livestock animal, are bred worldwide, providing humans with high-quality protein from milk [11], and have been long-neglected as a potential model to explore the maternal microbiome and early microbial colonization [12]. Dairy cattle have unique advantages over the inherent limitations of conventional rodent models, including a similar pregnancy cycle (280 days), number of births (one or twins), and closer biogeographic characteristics and phylogeny to humans [13], investigation of which may provide insights into the maternal microbiome and its transfer to offspring. In addition, the effect of maternal microbiota might be amplified in ruminants because their rumen stores a higher abundance and more diverse microbiota for fermenting plant fiber into volatile fatty acids (VFAs), which provides 70–80% of the energy requirements for the maintenance and production of ruminants [14, 15]. Hence, to further improve the efficiency of agricultural production, the rumen microbial community and its functions in adult cows have been studied extensively [16]. Different genetic backgrounds combined with nutritional strategies promote the differentiation of rumen microbiota in dairy cows and show remarkable differences in production phenotypes (high or low milk yield) [17], implying the characteristics of the maternal microbiome might also influence the early colonization of offspring. However, the use of dairy cattle models to explore the transmission characteristics of maternal microbiota to offspring is lacking.

In this study, we successfully screened an excellent production phenotypic dairy cow population (high milk yield) with a specific GIT microbiota that was distinguished from the prevalent phenotype (low milk yield) using enterotype analysis. We also used a multi-omics approach to further track the characteristics of the neonatal microbiome from the two phenotypic dairy cow populations and determined the variations and association of maternal and neonatal microbiomes corresponding to the different phenotypes of cows.

Results

Sample characteristics

A total of 308 pregnant dairy cows and their newborn calves from a commercial farm were enrolled. The corresponding feces, rumen contents, and blood samples from each cow were collected immediately after calving. The meconium and blood of the calves were collected before they were fed colostrum (within 30 min). We used 16S rRNA sequencing to detect the microbial community and diversity of rumen contents and fecal samples from all cows. Metagenomic shotgun sequencing and metabolomic analyses were performed on 28 pairs ($n=14$) of samples from cows to calves (rumen content, feces, meconium, and blood).

Identification of GIT microbial characteristics of dairy cows with excellent phenotype using enterotype analysis

Enterotyping is considered an effective method for dividing populations and revealing a general overview of inter-individual differences in the gut microbial community [18]. For the ruminal microbiota of cows, according to the Calinski Harabasz (CH) index of partitioning around medoids (PAM), the microbial profiling demonstrated an optimal number of two clusters ($k=2$) that showed the greatest robustness (Fig. 1A). These two clusters were defined as RUE1 and RUE2 (Figure S1A). Although we did not observe an obvious difference in alpha diversity between RUE1 and RUE2 (Fig. 1B), the microbial composition was distinctly different between these two groups. *Lachnospiraceae_NK3A20_group* and *Christensenellaceae_R-7_group* were shared dominant genera in the two groups (the relative abundance in both groups > 5%) (Figure S1C; Figure S2A). According to the linear discriminant analysis effect size (LEfSe) (LDA > 3, $P<0.05$), a total of 36 taxa were identified as signatures in the RUE1 and RUE2. Specifically, 13 signature taxa were enriched in RUE1 including the well-known functional genera *Prevotella*, *Rikenellaceae_RC9_gut_group*,

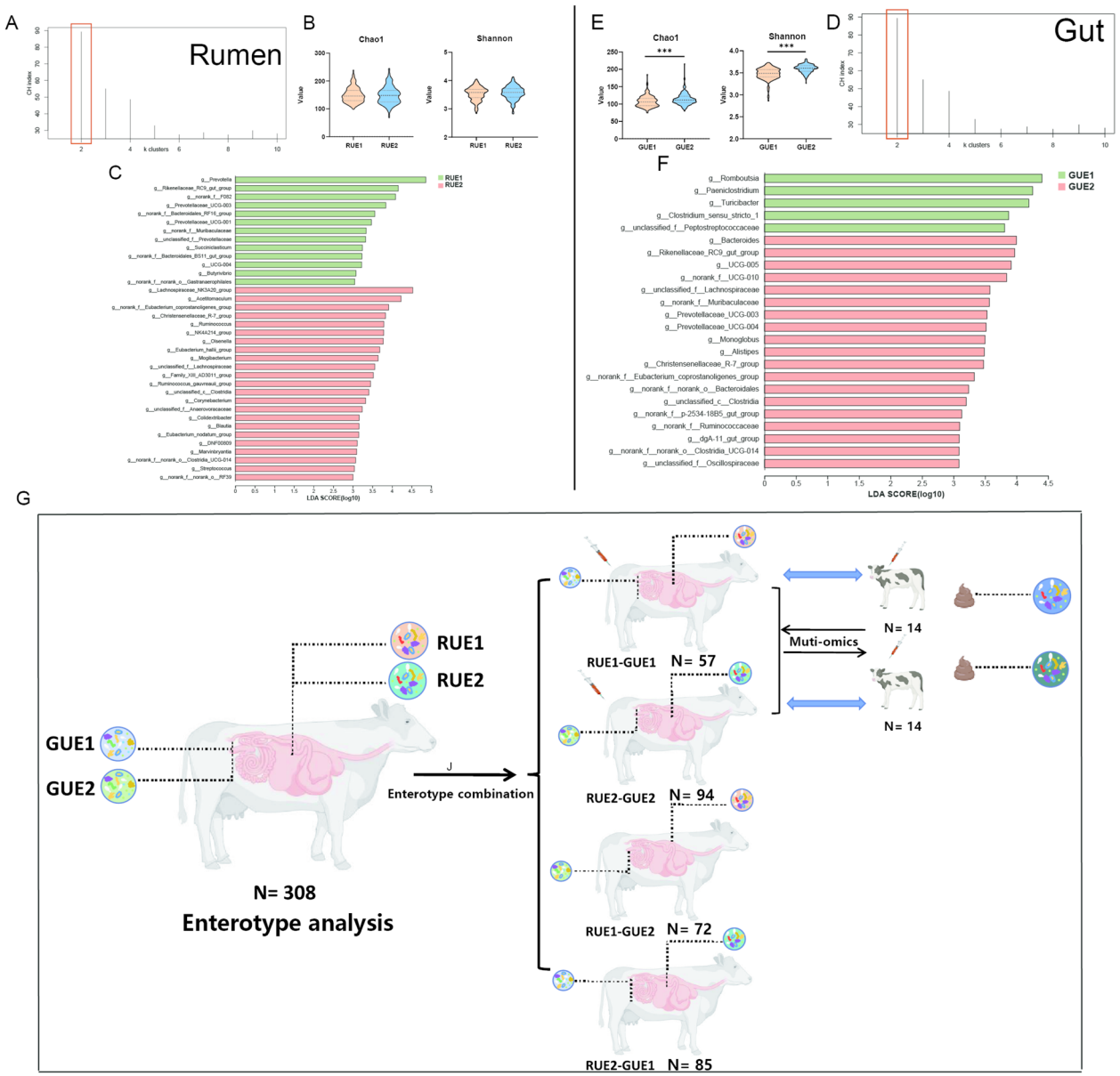

Fig. 1 Enterotype analysis and combination of rumen and gut of dairy cows. **A** CH index of rumen enterotype robustness. **B** Comparison of microbial richness (Chao1 index) and diversity (Shannon index) between the two rumen clusters. **C** Identification of signature genera of the two rumen clusters using LEfSe. **D** CH index of gut enterotype robustness. **E** Comparison of microbial richness (Chao1 index) and diversity (Shannon index) between the two gut clusters. **F** Dominant genera across all rumen and gut samples. **G** schematic of workflow

Succiniclasticum, and *Butyrivibrio*. In contrast, *Lachnospiraceae_NK3A20_group*, *Acetitomaculum*, and *Ruminococcus* were the signature functional genera in RUE2 (Fig. 1C). Combined with the production performance data for cows, we found the 305-day and peak milk yields of cows in RUE1 were significantly higher than those in RUE2 ($P < 0.05$) (Table S1). The other indicators showed no significant differences between the two groups. Similarly, the fecal microbiota was divided into two clusters ($k = 2$) (Fig. 1D), which showed the highest stability. These two clusters were defined as GUE1 and GUE2 (Figure S1B). The fecal microbiota of GUE2 showed higher alpha diversity, including higher Chao1 and Shannon indices, than that of GUE1 ($P < 0.05$) (Fig. 1E). *Rikenellaceae_RC9_gut_group*, *Oscillospiraceae_UCG-005,* and *Romboutsia* were the shared dominant bacteria in the two groups (the relative abundance in both groups > 5%) (Figure S1C; Figure S2B). Moreover, according to the

LEfSe (LDA > 3, $P < 0.05$), *Romboutsia* was identified as the signature genus in GUE1 ($P < 0.05$), and *Rikenellaceae_RC9_gut_group* and *Oscillospiraceae_UCG-005* were the signatures in GUE2 (Fig. 1F). We compared the production performances of GUE1 and GUE2, and no significant differences were detected (Table S2). Moreover, although most current studies have revealed a close relationship between rumen microbiota and production performance, the hindgut microbiota still plays an important role in regulating the health and metabolism of the host, which might indirectly affect cow production. Therefore, we further combined the enterotypes of the rumen and hindgut in pairs to divide the cows into four groups designated RUE1-GUE1, RUE1-GUE2, RUE2-GUE1, and RUE2-GUE2, which may contribute to the further screening of dairy cow populations with excellent production traits (Fig. 1G). As expected, the cows in RUE1-GUE1 showed the best production performance, including the highest 305-day, yearly, peak, and average daily milk yields compared to the other groups ($P < 0.05$). In addition, the number of parities in RUE1-GUE1 was higher than those in RUE1-GUE2 and RUE2-GUE1 ($P < 0.05$), and the days of gestation showed the opposite trend ($P < 0.05$) (Table 1).

Concordance of microbial variation between neonatal calves and cows with different phenotypes

To further explore the potential effects of different phenotypes of cows on the microbiome of their neonatal calves, we selected 14 cows and their neonatal calves from RUE1-GUE1 (named EPG cow/calf) and RUE2-GUE2 (named PPG cow/calf), respectively, for subsequent multi-omics analyses (Fig. 1G). Consistent with the results of the large group, the EPG cows showed greater production performance than the PPG cows ($P < 0.05$), and no differences were observed in individual characteristics between these two groups which avoided the interference of other factors on milk yield and microbiota (Table 1). For beta diversity, the geographical divisions of the different GIT microbiomes (rumen and hindgut) in the cows were distinct ($P < 0.05$) (Figure S3A). In addition, the microbial communities of the cows and their neonatal calves in the EPG were remarkably different from those in the PPG ($P < 0.05$) (Figure S3A; Figure S4). Regarding alpha diversity, the rumen microbiota showed the highest Chao1 index, and the meconium microbiota had the lowest Chao1 index ($P < 0.05$), whereas no difference was observed between the EPG and PPG cows/calves (Figure S5A). In addition, the EPG calves showed a higher Shannon index than the PPG calves ($P < 0.05$) (Figure S5B). In terms of microbial composition at the species level, *Lachnospiraceae bacterium*, *Clostridia bacterium*, *Bacteroidales bacterium*, *Oscillospiraceae bacterium*, and *Prevotella sp.* were dominant in the rumen of both groups, among which *Bacteroidales bacterium* and *Prevotella sp.* showed a higher abundance in the EPG (Figure S6A). In the feces, the dominant species were *Oscillospiraceae bacterium*, *Clostridia bacterium*, and *Lachnospiraceae bacterium*, and the abundance of *Oscillospiraceae bacterium* was higher in the PPG (Figure S6B). The most distinct difference was observed in the meconium. Although *unclassified_Achromobacter*, *Achromobacter insuavis*, *Achromobacter deleyi*, and *Achromobacter xylosoxidans* were the dominant species in the EPG and PPG, their abundances were remarkably higher in the PPG than in the EPG (Figure S6C).

The Venn diagram shows that, compared to the feces, the rumen shares more bacterial species with the meconium in both groups (9320 vs. 7396 in the EPG; 5157 vs. 3931 in the PPG) (Figure S3B). In the EPG, the number of

Table 1 Comparison of phenotypes of cows among the four groups

Items	mean				SEM	*P*
	RUE1-GUE1	RUE1-GUE2	RUE2-GUE1	RUE1-GUE1		
Performance						
305-day milk yield/kg	10,688.14^{a}	9329.19^{b}	9467.81^{b}	9665.27^{b}	124.50	0.002
Yearly milk yield/kg	10,227.78^{a}	8713.34^{b}	8897.37^{b}	9257.02^{b}	129.35	0.001
Peak milk yield/kg	48.57^{a}	42.95^{b}	44.64^{b}	44.79^{b}	0.62	< 0.001
The day of peak milk	55.56	57.69	55.19	56.87	1.57	0.940
Average daily milk yield/kg	35.86^{a}	32.19^{b}	31.83^{b}	32.65^{b}	0.42	0.009
Individual characteristics						
Parity	2.07^{a}	1.51^{c}	1.69^{bc}	1.96^{ab}	0.05	< 0.001
Gestation period/day	275.35^{b}	277.72^{a}	277.55^{a}	275.66^{b}	0.29	0.003
Weight/kg	760.61	725.60	734.02	738.08	4.81	0.106
Body condition score	3.36	3.38	3.36	3.36	0.02	0.962

^{a,b} Values in a row with no common superscripts differ significantly ($P < 0.05$)

core species shared by the rumen, feces, and meconium was higher than that in the PPG (7302 vs. 3889). SourceTracker, a tool used to identify the proportion of target microbial communities from other sources, was used to detect the maternal origin of meconium microbiota. Consistent with the Venn results, the rumen was a more important microbial source for the meconium microbiota than the feces, and the meconium inherited more microbiota from the rumen and feces in the EPG than in the PPG (> 50% vs. < 50%) (Figure S3C). Furthermore, considering the significant differences in both maternal and neonatal microbiomes between the EPG and PPG, which might be influenced by the production performance of cows. We further explored the consistency of microbial variation between cows and their calves through the comparison of the two groups (EPG vs PPG) at the species level. We first identified the significantly enriched bacteria ($P < 0.05$) in the gut of cows and their calves from the EPG and PPG respectively, and then calculated the ratio of the abundance of these bacteria in the two groups (EPG/PPG). Finally, we screened out the microbial taxa with the same variation trend between the maternal and neonatal microbiota (the ratio of abundance was both > 1 and < 1). The results showed that more enriched bacteria in the rumen and feces continued from the cow to their offspring in the EPG. Specifically, *Prevotella* and *Bacteroides* were the dominant bacteria enriched in the rumen and meconium of the EPG, including *P. bryantii*, *P. copri*, *P. mizrahii*, *P. sp.*, *B. caccae*, *B. eggerthii*, *B. fragilis*, *B. heparinolyticus*, and *B. ovatus*. In addition, *Bifidobacterium animalis* and *Bifidobacterium pseudolongum* were the most prevalent taxa from cows to calves in the EPG. In contrast, *Actinomyces succiniciruminis*, *Cupriavidus taiwanensis*, *Erysipelotrichaceae bacterium*, and *uncultured_Faecalibacterium sp*. were enriched in the rumen and meconium of the PPG (Fig. 2A). In the feces and meconium, we observed that *Bifidobacterium*, *Clostridium*, and *Romboutsia* were dominant from cow to calf in the EPG, including *B. adolescentis*, *B. choerinum*, *B. merycicum, B. pseudocatenulatum*, *B. pseudolongum*, *C. beijerinckii*, *C. cuniculi*, *C. perfringens*, *C. sp.*, *R. ilealis*, *R. lituseburensis*, and *R. sp.* Whereas, *Burkholderia cenocepacia* and *Sutterella wadsworthensis_CAG:135* showed higher abundances in the feces and meconium of the PPG (Fig. 2B). In view of the specificity of the rumen microbiome with different production performances, and its close relationship with the neonatal meconium, the Hungate1000 collection 16, a database of bacterial and archaeal species isolated and cultured from the rumen of a variety of ruminants, was used to further deepen our understanding of microbial taxa identification and function in the rumen at the strain level (Fig. 2C). A total of 361 microbial strains were identified in the rumen, of which 38 showed significant differences in abundance ($P < 0.05$). Among them, 28 strains belonging to *Prevotella* were abundant in the EPG, including *P sp. NE3005*, *MA2016*, *P. ruminicola D31d*, *P. ruminicola KHT3*, and *P. ruminicola BPI-34*, which favor the utilization of xylan, pectin, starch, and protein and the production of acetate, propionate, and succinate. Three strains, *Micrococcineae bacterium KH10*, *Lachnospiraceae bacterium FE2018*, and *Clostridium algidicarnis B3* were enriched in the PPG but did not have the above metabolic functions.

The variation of microbial function in cows may be inherited by neonatal calves

Metagenomic functional analysis was performed to characterize the functions of gut microbes in cows and their calves. The pathways involved in substance metabolism showed significant differences between the EPG and PPG. Specifically, microbial functions in the rumen of EPG cows showed lower abundances of amino acid metabolism pathways ($P < 0.05$), including "alanine, aspartate, and glutamate metabolism," "histidine metabolism," "valine, leucine, and isoleucine biosynthesis," "arginine biosynthesis," "tyrosine metabolism," and "phenylalanine metabolism". However, the pathways related to vitamin metabolism, such as "vitamin b6 metabolism," "folate biosynthesis," "riboflavin metabolism," "retinol metabolism," and "biotin metabolism" were higher in abundance ($P < 0.05$). In addition, the lipid metabolism pathways of "sphingolipid metabolism," "fatty acid biosynthesis," "steroid biosynthesis," and "linoleic acid metabolism" showed similar trends ($P < 0.05$). Of note, in energy metabolism, the pathway of "oxidative phosphorylation" was higher and "Methane metabolism" was lower in the EPG ($P < 0.05$) (Fig. 3A). Compared to the rumen, the fecal microbiome had fewer significantly different pathways between the two groups. Contrary to the lower abundance of amino acid metabolism in the rumen, three amino acid metabolic pathways were enriched in the feces of EPG cows, including "alanine, aspartate, and glutamate metabolism," "phenylalanine metabolism," and "valine, leucine and isoleucine biosynthesis" ($P < 0.05$). All the pathways in carbohydrate metabolism were lower and four pathways related to vitamin metabolism were higher in the feces of EPG cows including "folate biosynthesis," "pantothenate and coa biosynthesis," "porphyrin metabolism," and "riboflavin metabolism" ($P < 0.05$) (Fig. 3B). In the meconium of the calves, more pathways with significant differences were observed between the two groups. Consistent with the rumen, most pathways involved in amino acid metabolism showed a lower abundance in the EPG calves ($P < 0.05$). Conversely, pathways related to glycan biosynthesis

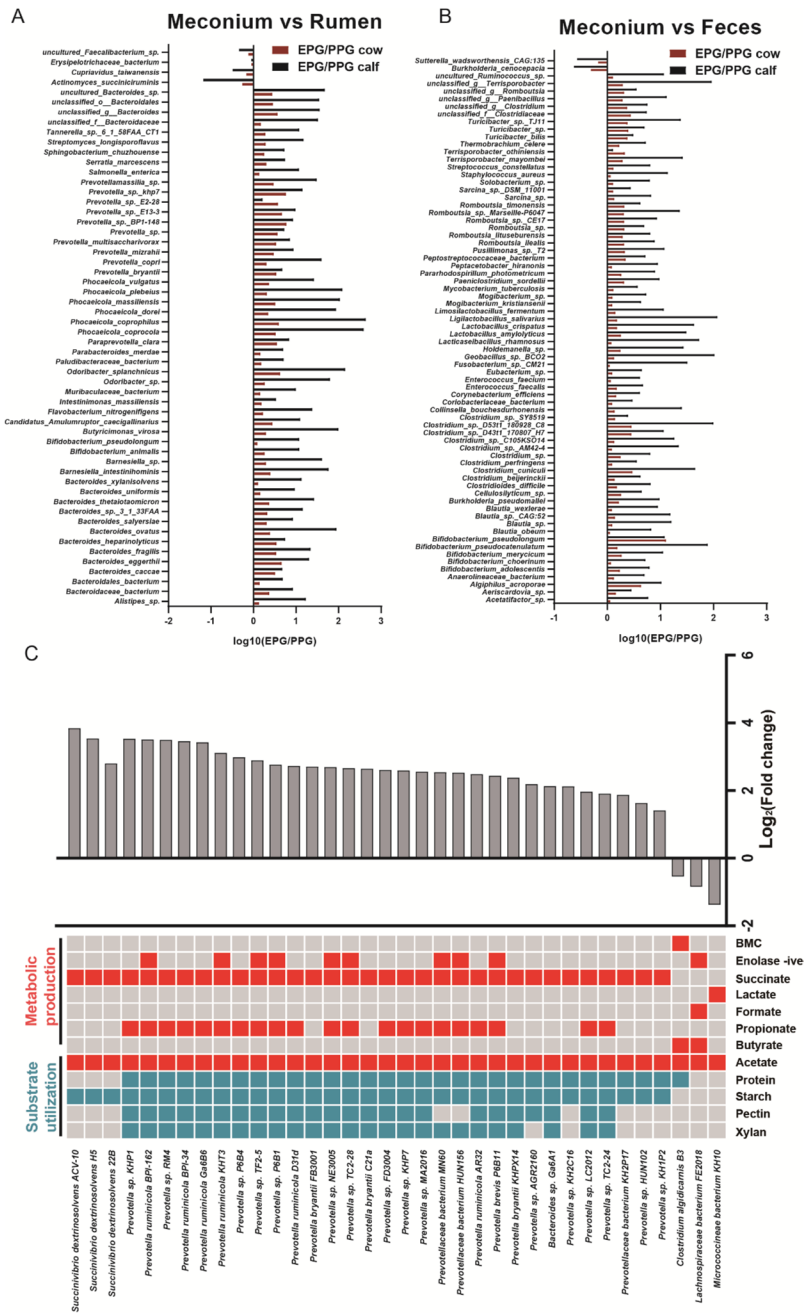

Fig. 2 Microbial consistency between meconium, rumen and feces. **A** Species variation concordance between meconium and maternal rumen microbiota. **B** Species variation concordance between meconium and maternal feces microbiota. EPG/PPG = the ratio of microbial abundance between EPG and PPG. **C** Identification of cultured strains from rumen in the Hungate1000 collection

and metabolism were enriched. Moreover, several important pathways of carbohydrate and lipid metabolism were more abundant in the EPG calves ($P < 0.05$), including "starch and sucrose metabolism," "galactose metabolism," "glycolysis/gluconeogenesis," "fructose and mannose metabolism," "pyruvate metabolism," "sphingolipid metabolism," and "secondary bile acid biosynthesis" (Figure S7A).

Next, we tracked the microbial hosts at the species level for six major metabolic functions using metagenomic assembly. In the rumen, *Lachnospiraceae bacterium* and *Oscillospiraceae bacterium* were the dominant

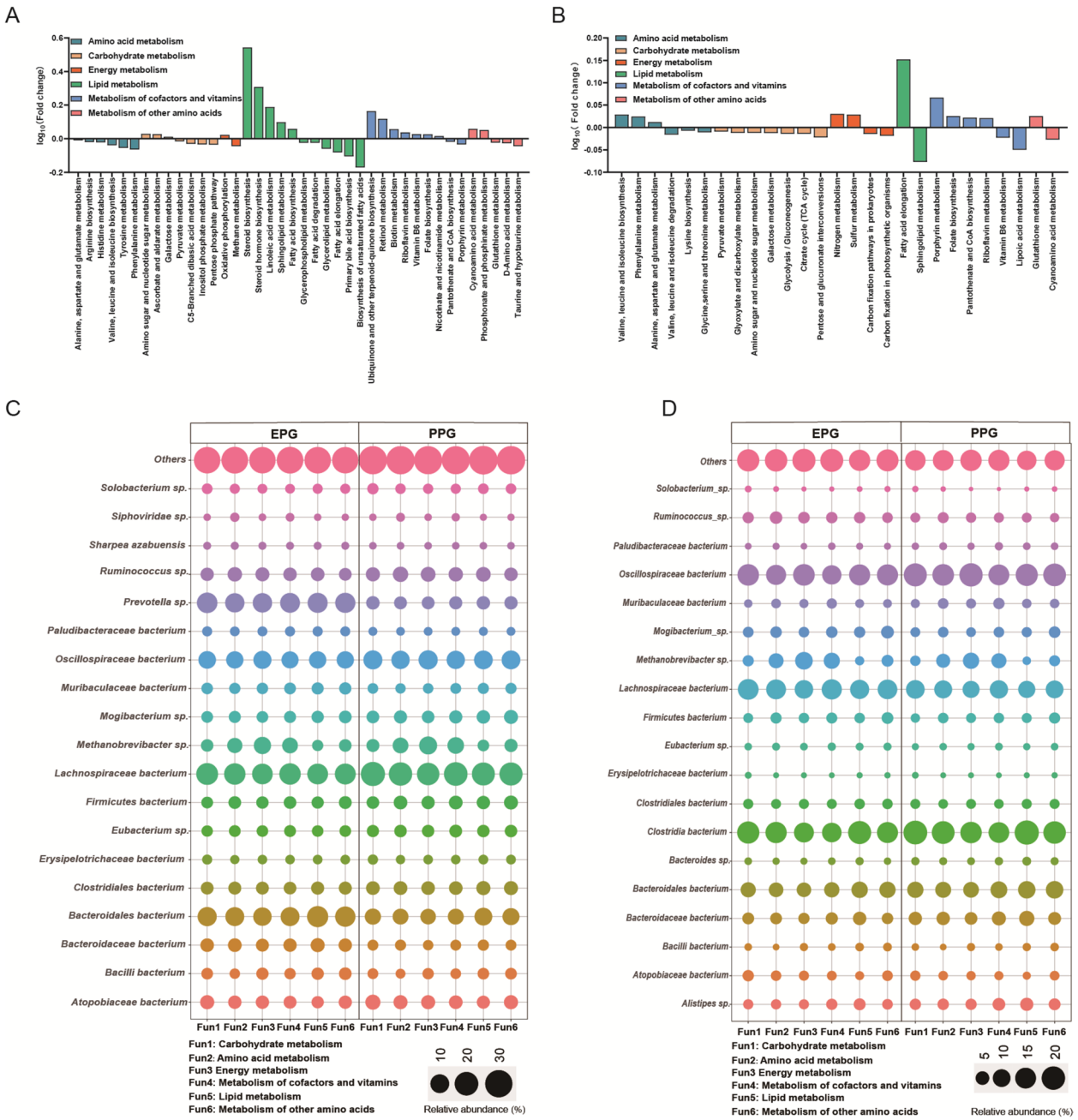

Fig. 3 Differences between the EPG and PPG in KEGG metabolic function attributed to the microbiome of rumen and feces. **A** Significantly different KEGG pathways related to metabolism of ruminal microbiota in the EPG and PPG. **B** Significantly different KEGG pathways related to metabolism of fecal microbiota in the EPG and PPG. **C** Bubble plots depicting the difference of microbial hosts of metabolic functions at the species level in the rumen. **D** Bubble plots depicting the differences between the metabolic functions of microbial hosts at the species level in the feces

bacteria in both groups, and the predicted abundances of *Bacteroidales bacterium* and *Prevotella sp.* were higher in the EPG than in the PPG (Fig. 3C). In the feces, *Oscillospiraceae bacterium*, *Clostridia bacterium*, and *Lachnospiraceae bacterium* were the top three bacterial hosts in the two groups; the abundance of the first two bacteria was higher and that of the third was slightly lower in the EPG (Fig. 3D). In the meconium, *Achromobacter deleyi*, *Achromobacter insuavis*, and *unclassified_ Achromobacter* were the dominant hosts in both groups and were all more abundant in the PPG. Conversely, several microbial hosts showed higher abundance in the EPG, including

Bifidobacterium pseudocatenulatum, *Lachnospiraceae bacterium*, *Oscillospiraceae bacterium*, and *Bacteroidales bacterium* (FigureS7B).

Moreover, we identified the differential genes between the two groups by comparing their abundance ($P < 0.05$). In total, 1729 DEGs were detected in the rumen, of which 682 were enriched in the EPG and 1047 in the PPG. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of the DEGs in each group were conducted using the KEGG Mapper. We identified and described the predominant metabolic functions regulated by the ruminal microbiome in cows. As shown in Fig. 4A, genes related to starch and cellulose degradation were upregulated in the EPG, including *E3.2.1*, *bglX*, *bglB*, and *susB*, whereas the genes *surA*, *INV*, and *HK* involved in sucrose degradation were downregulated. In the subsequent glycolytic process, the abundance of most related genes (*galM*, *glk*, *apgM*, *ppdK*, *korA*, and *korB*) increased, whereas only a few related genes showed the opposite trend, including *pfkB* and *FBA*. Notably, the expression of genes involved in amino acid metabolic pathways was generally suppressed. Moreover, the synthesis of long-chain fatty acids, propanoate, and acetate was activated in the EPG, including upregulated genes such as *sdhA*, *sdhB*, *ACSS_2*, *MUT*, *MCEE*, *scpB*, *FabD*, *FabH*, *FabA*, and *FabZ*. On the contrary, genes involved in methane and butanoate metabolism (e.g., *fadE*, *cdhE*, *ACDS*, *aarC*, *paaH*, *crt*, and *ydlF*) decreased in abundance. Unfortunately, only 156 DEGs (133 enriched in the EPG and 23 enriched in the PPG) were detected in the feces of cows, and the metabolic pathways that were highly enriched by these DEGs were not observed. Based on the above results, to evaluate the vertical transmission effect of the functional group of the maternal microbiome, we screened DEGs with the same expression trend in the meconium and rumen and further performed functional enrichment analysis (Fig. 4B). Consistent with the rumen microbiome, the degradation of starch and cellulose was activated and butanoate metabolism was inhibited in the EPG meconium. In addition, N-glycan biosynthesis and glycosphingolipid biosynthesis were also enriched by related DEGs in both the rumen and meconium of the EPG.

Transmission characteristics of resistome from dairy cows to neonatal calves

To understand the characteristics of resistome transmission from cows to calves, we detected resistome profiles

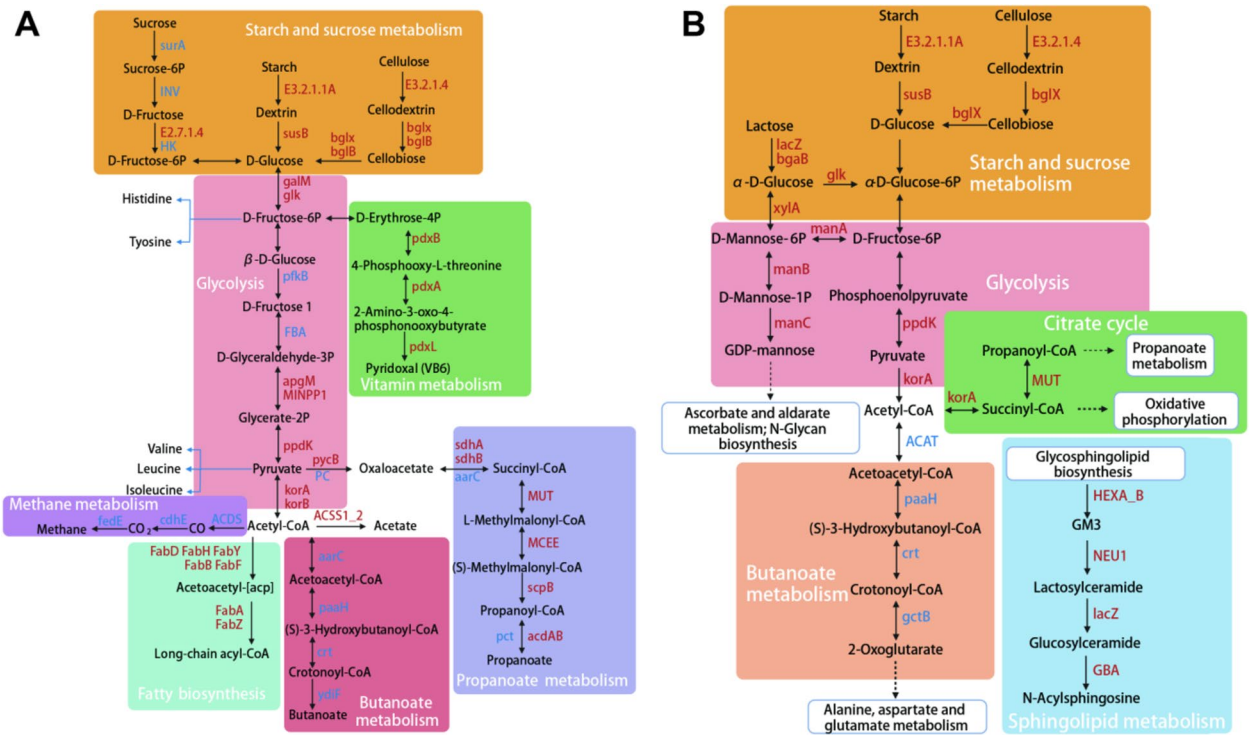

Fig. 4 Metabolic pathways enriched by DEGs between the EPG and PPG. **A** Enriched metabolic pathways involved in starch and sucrose metabolism, glycolysis, vitamin metabolism, VFA metabolism, and methane metabolism in the rumen microbiome. **B** Enriched metabolic pathways involved in starch and sucrose metabolism, glycolysis, N-glycan biosynthesis, and glycosphingolipid biosynthesis shared by the rumen and meconium microbiome

in the maternal and neonatal microbiomes in each group. We did not observe a significant difference in total antibiotic resistance gene (ARG) abundance in the feces between the EPG and PPG. The total ARG abundance in the rumen and meconium was higher in the PPG than in the EPG ($P < 0.05$) (Fig. 5A). The overall ARG structure in the meconium of calves also showed a similar pattern to the rumen and feces in the cows in each group (Fig. 5B). We further detected shared ARGs in the rumen, feces, and meconium of the EPG and PPG. More shared ARGs were observed in the EPG than the PPG (490 vs. 363), which was consistent with the results for microbial taxa (Fig. 5C). The ARGs conferring resistance to glycopeptide, multi-drug, and tetracycline were highly abundant in both groups. Notably, the advantage in the abundance of maternal ARGs was further expanded in the meconium of calves, and the trend of expansion was more obvious in the PPG than the EPG (Fig. 5D). Furthermore, mobile genetic elements (MGE) are an important driving force that mediate horizontal gene transfer within and between bacteria and are also a key reference factor for the investigation of drug resistance gene transfer in bacteria. In this study, we detected the MGE profile in the cow-to-calf transfer chain by annotating four databases: ICEberg, ISFinder, National Center for Biotechnology Information (NCBI) Plasmid RefSeq, and Integrall.

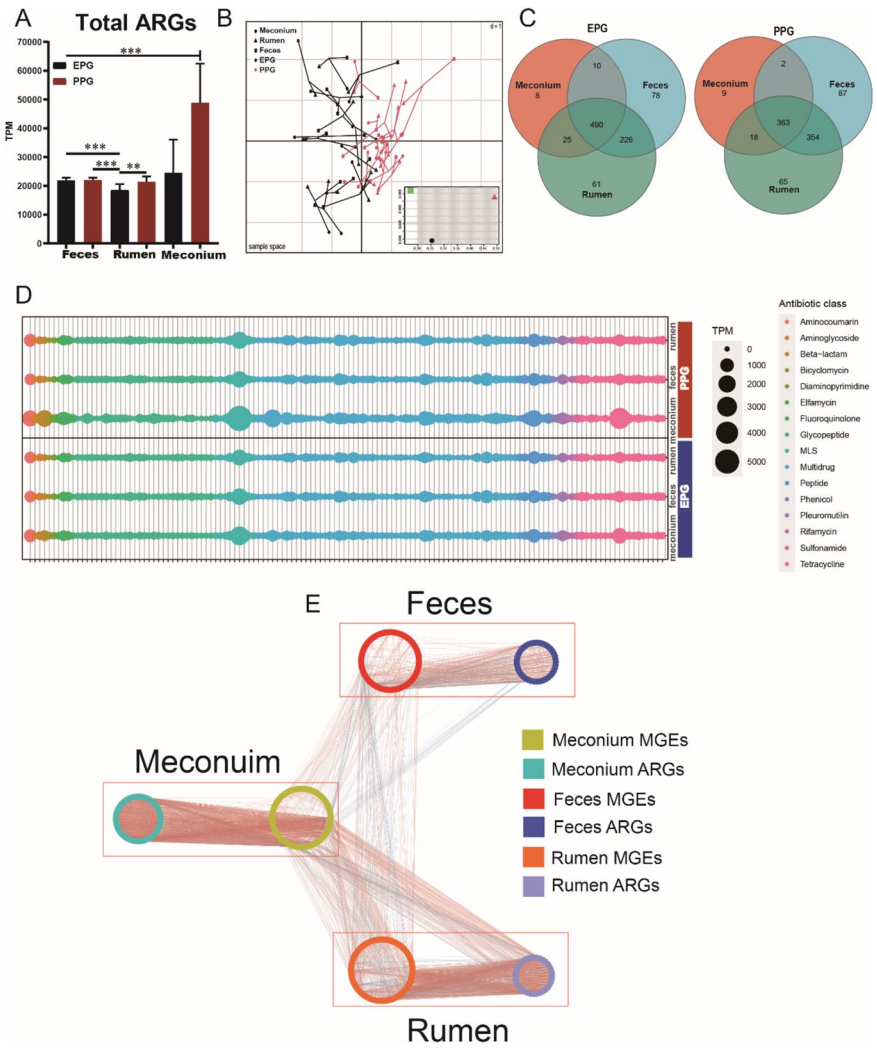

Fig. 5 Vertical transmission of resistome from the rumen and feces of cows to calf meconium. **A** Total abundance of ARGs in the rumen, feces, and meconium. **B** Multiple co-inertia analysis of rumen, feces, and meconium resistomes in the EPG and PPG. **C** Wayne diagram showing the ARG intersection of rumen, feces, and meconium in the EPG and PPG. **D** Presence of dominant ARGs in the rumen, feces, and meconium of the EPG and PPG. **E** Co-occurrence network revealing associations of ARGs and MGEs in the rumen, feces, and meconium. TPM: Transcripts per million; ARG: Antibiotic resistance gene; MGE: Mobile genetic elements

The abundance of total MGEs and plasmids was higher in the rumen and meconium of the PPG than the EPG ($P < 0.05$) (Figure S8A and S8E), and the total MGEs from the ICEberg and Integral databases within the meconium of the PPG also showed a higher abundance compared to other groups, suggesting a similar expression pattern to that of the ARGs (Figure S8B and S8C). For ISFinder, the MGEs were higher in the rumen of the EPG than the PPG, and the MGEs in the meconium of both groups were higher than those in the rumen and feces (Figure S8D). To evaluate the horizontal transfer frequency of ARGs depending on MGEs and their vertical transmission effect from cow to calf, a co-occurrence network was constructed to reveal the associations between ARGs and MGEs in the rumen, feces, and meconium (Fig. 5E). The most complex correlations between ARGs and MGEs in the meconium were observed in the rumen and feces. In addition, compared with feces, ARGs, and MGEs in the rumen showed a stronger correlation with MGEs in the meconium, suggesting a closer relationship of the resistome between the rumen and meconium

Identification and variation of core metabolites in the maternal and neonatal transfer chain

We also determined the metabolomics of the rumen, feces, and blood of cows and the meconium and blood of calves to describe maternal and neonatal metabolic characteristics. The Venn diagram analysis showed that 517 and 519 metabolites were co-annotated by the five sample types of EPG and PPG, respectively. Moreover, these metabolites were identified in more than half of the samples in each group, which were defined as the core metabolites in the EPG and PPG owing to their widespread presence in calves and cows. Of these two sets of core metabolites, 500 metabolites were shared, accounting for the vast majority of both sets (500/517; 500/519), suggesting that the division of core metabolites in the cow-calf transmission chain was highly stable and was not disturbed by maternal phenotypic differences (Fig. 6A). However, a significant difference in the abundance of metabolites was observed between the EPG and PPG. A total of 394 (53 upregulated and 341 downregulated), 468 (313 upregulated and 155 downregulated), and 1885 (641 upregulated and 1244 downregulated) differential metabolites were identified in the rumen, feces, and meconium, respectively, between the EPG and PPG ($P < 0.05$) (Figure S9A–C). We further screened the core metabolites with significant differences in the rumen, feces, and meconium, and conducted MetOrigin analysis to better characterize the origin of these metabolites [19]. In the rumen, 16 metabolites were derived from the host, 27 from the microbiota, and 14 were shared between the two (Figure S10A). In the feces, 12 metabolites were derived from the host, 29 from the microbiota, and 11 were shared between the two (Figure S10B). A greater number of metabolites were detected in the meconium; 81 metabolites were derived from the host, 151 from the microbiota, and 71 were shared between the two (Figure S10C). In addition, functional enrichment and network analysis were performed to character the relationships between these differential core metabolites and their enriched pathways in the rumen, feces, and meconium respectively. Of note, since host-microbial nutrient metabolism interactions were the focus of this study, the co-occurrence networks displayed the main functional pathways and metabolites involved in the metabolism of carbohydrates, amino acids, volatile fatty acids, and their derivates. As a result, the rumen metabolites were mainly enriched in the pathways of "Tryptophan metabolism," "Sphingolipid metabolism," "D-Amino acid metabolism," "Fatty acid biosynthesis," and "Vitamin B6 metabolism" (Fig. 6B) including upregulated L-serine and octanoate and downregulated L-glutamine and 2-aminobenzoic acid (Fig. 6D). In the feces, the pathways of "alpha-linolenic acid metabolism," "fatty acid biosynthesis," "cysteine and methionine metabolism," "sphingolipid metabolism," and "glycine, serine and threonine metabolism" were enriched by the upregulated 4-hydroxysphinganine, 5′-Methylthioadenosine, and 2-oxo-PDA and downregulated octanoate (Figure S11A, B). The metabolites in the meconium formed a more complex functional annotation network, suggesting that the variation in the microbial metabolic profile was magnified in neonatal calves. The upregulated L-glycine, L-glutamine, 2-oxoarginine, 5′-methylthioadenosine, and lactic acid and downregulated L-serine, L-proline, and diaminopimelic acid were enriched in the pathways of "arginine and proline metabolism," "cysteine and methionine metabolism,"

(See figure on next page.)

Fig. 6 Metabolic profile of serum and microbiome from cow to calf. **A** Wayne diagram showing the metabolic intersection of the rumen, feces, meconium, cow serum, and calf serum in the EPG and PPG. **B** Metabolic pathway enrichment analysis of differential core metabolites in the rumen. **C** Metabolic pathway enrichment analysis of differential core metabolites in the meconium. **D** Pathway enrichment analysis of metabolites and their association network in the rumen. **E** Pathway enrichment analysis of metabolites and their association network in the meconium. The major metabolic pathways are marked in orange nodes and the nodes of signature metabolites are marked in red (enriched in the EPG) or blue (enriched in the PPG) in the association network

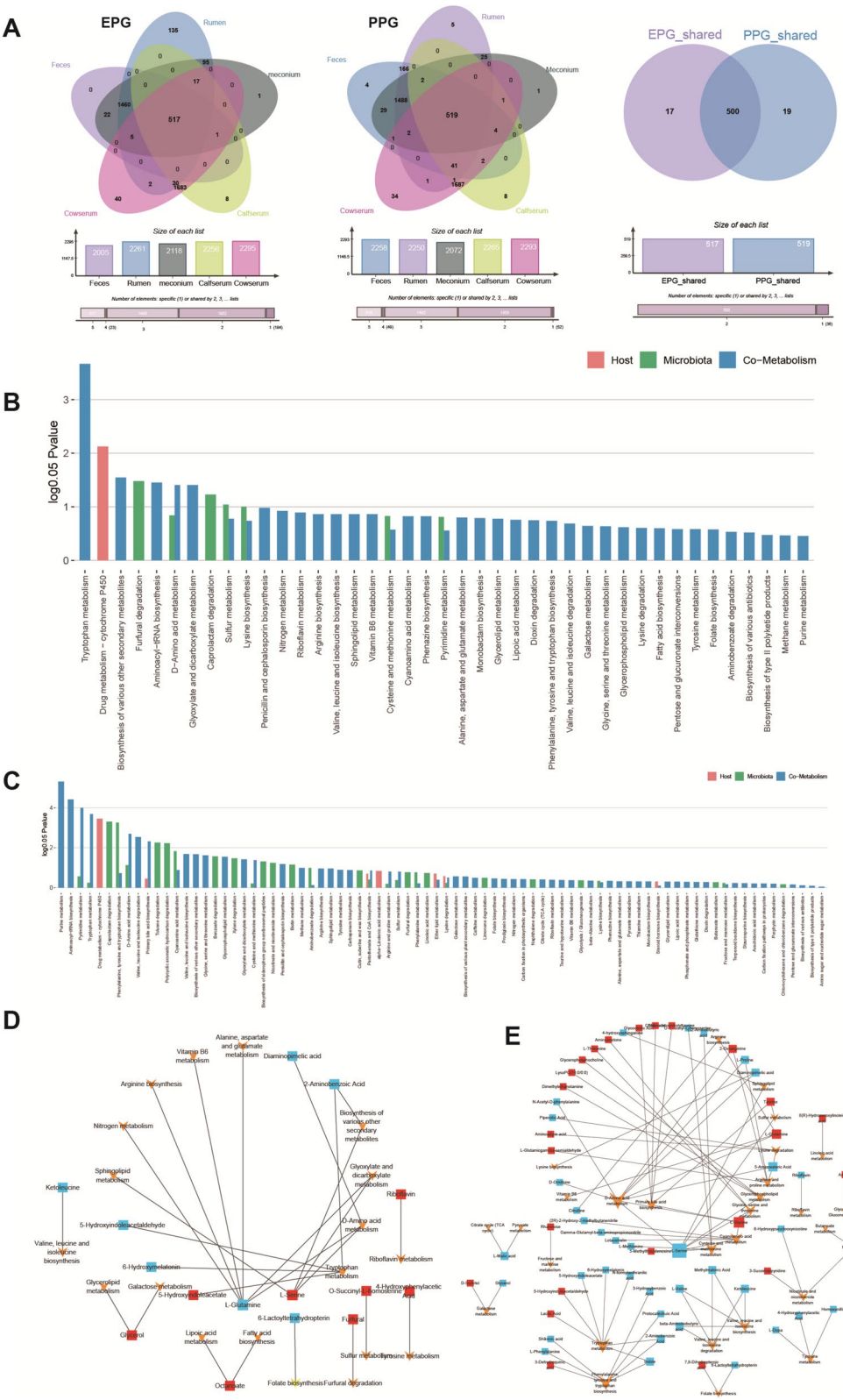

Fig. 6 (See legend on previous page.)

"tryptophan metabolism," and "D-amino acid metabolism" (Fig. 6C, E).

Discussion

The maternal microbiome is an important factor that shapes the neonatal gut microbial community and its function. To our knowledge, this is the first study to integrate large-sample enterotype analyses to classify the production phenotype of cows with characteristic GIT microbiota, and further explore the vertical transfer effect of the microbiome in cows with different phenotypes to their corresponding calves using multi-omics analyses.

Consistent with previous studies [17, 20], we observed that cows with different production performances had distinct rumen microbial communities. The rumen, a unique digestive organ of ruminants, is the most important site for nutrient metabolism and relies on the complex rumen microbial ecosystem. In this study, the ruminal enterotypes of high-yield dairy cows were dominated by several nutritional decomposers, including *Prevotella*, *Succiniclasticum*, and *Butyrivibrio. Prevotella* is an efficient utilizer of carbohydrates, including fiber and non-fiber, which can be converted into VFAs [21]. *Butyrivibrio* is also a VFA producer that degrades dietary fibers. *Succiniclasticum* is highly involved in succinic acid metabolism, and succinic acid, as an intermediate, mediates the synthesis of multiple downstream nutrients, including VFAs [22]. VFAs, instead of glucose, as the primary energy source in dairy cows distinguish them from monogastric hosts [23]. The higher concentration of VFAs produced by acidogenic bacteria enriched in the rumen can not only meet the maintenance needs of dairy cows but also maximize their productivity. In addition, although the microbiota in the hindgut is not directly related to the production performance of dairy cows, and we did not observe a difference in milk yield in cows with different fecal enterotypes, it is undeniable that homeostasis of the hindgut microbiota plays an important role in regulating host lipid metabolism [15], oxidative stress [24], disease, and immunity, which might indirectly affect the short-term or long-term productivity of dairy cows. Consistently, according to enterotype pairing, the synergistic effects of rumen and hindgut microbiota were associated with the identification of a population of cows with an excellent phenotype in this study, suggesting the potential effect of the hindgut in the regulation of dairy cow production.

Like other mammals, most initial colonizers in the gut of newborn calves originate from maternal sites [25], among which the rumen might be the most important source because of its complex and diverse microecosystem [26]. In previous studies, the core taxonomic group of the rumen microbiota was shown to be heritable [27] and strongly correlated with host traits, including rumen fermentation, milk production efficiency, and blood metabolites [28]. However, these heritable microbes were based on crossbreed comparisons or associations of the host genotype [28, 29], rather than directly comparing the microbiome characteristics between cows and their offspring. In this study, compared with feces, we observed a closer correlation of microbial taxa between the rumen and meconium in both groups, especially in the EPG, where > 50% of microbes in the calf meconium originated from the rumen of the dam. As a typical ruminant, the ruminant behavior of dairy cows contributes to the prevalence of rumen microbiome in their oral cavity. A previous study has demonstrated that the microbial composition and taxa in the saliva of dairy cows were highly similar to those in their rumen [30]. During this experiment, we observed that cows habitually licked their newborn calves after delivering. Hence, we speculated this behavior might promote rumen microbiota transmission from mother to calf via the saliva-mediated pathway. Furthermore, several human studies have reported that some of the early colonizing microbes in the gut of neonates also originated from the oral or respiratory tract of the mother [31, 32]. These evidences also lend support to our hypothesis. Moreover, in the rumen and feces of the EPG, many beneficial bacteria with high abundance were transferred to the meconium and maintained their dominance, including members of *Prevotella*, *Bifidobacterium*, and *Bacteroides*. *Prevotella* is an early colonizer that can persist in the gut of newborn ruminants for a long time. One study suggested that *Prevotella* might be a potential probiotic for preventing calf diarrhea [33]. Relying on an exclusive database of ruminant rumen microbiomes [34], we further identified several strains of *Prevotella* and *Succinivibrio* enriched in the EPG, suggesting that the favorable growth phenotype of cows might be driven by gut-specific *Prevotella* taxa. In addition, *Bifidobacterium* and *Bacteroides* reside in the gut from birth to adulthood of ruminants. They can also be used as biomarkers to determine the balance between gut microbial community and host inflammation [35]. This evidence implies the possibility of promoting the future productivity of offspring by the vertical transmission of beneficial microbiota in the gut of dairy cows.

Furthermore, we detected the profiles of microbial functions at the three sites between the EPG and PPG. In the rumen, the abundance of pathways related to amino acid metabolism was enriched, and several metabolic pathways were involved in Carbohydrate metabolism, Lipid metabolism, and the Metabolism of cofactors and vitamins in the EPG. As fatty acids are the main end products of rumen microbial metabolism and show

strong correlations with production performance [36], it is not surprising that lipid metabolism was enriched in the EPG. In addition, vitamin biosynthesis activity cannot be ignored. As an essential nutrient for the health and production of dairy cows, a previous study demonstrated that vitamin B biosynthesis was mainly performed by the rumen microbiome rather than the hindgut [37], which is consistent with our results, explaining the advantages of critical metabolic pathways in the EPG. Notably, methane metabolism appeared to be decreased, suggesting that more free hydrogen in the rumen tends to be synthesized by nutritional bacteria than by methane production in EPG cows [16, 38, 39]. In terms of feces, opposite to the rumen, amino acid metabolism including valine, leucine, and isoleucine biosynthesis, phenylalanine metabolism, and alanine, aspartate and glutamate metabolism were abundant in the feces of EPG cows. We also observed enriched pathways in the EPG during the metabolism of cofactors and vitamins. Combined with the functional group results of the rumen and feces, we speculated that the coordination and complementarity of region-specific functions of the GIT microbiome were the metabolic basis for shaping the excellent production phenotypes in EPG cows [11]. The functional profile of the meconium of EPG calves was more similar to that of the rumen, including the inhibition of amino acid metabolism enriched lipid metabolism, and Metabolism of cofactors and vitamins. In addition, most pathways involved in carbohydrate metabolism and glycan biosynthesis and metabolism were also abundant, implying that the functional group of the meconium microbiome was more consistent with that of the rumen and showed greater variation between the two groups.

Prediction of the microbial host bacteria executing metabolic functions showed *Prevotella sp.* and *Bacteroidales bacterium* were the specific hosts that executed the main metabolism in the rumen of EPG cows. In the feces, we did not observe a distinct difference in the microbial host between the EPG and PPG, which might be attributed to the shared dominant bacteria with similar abundances in both groups. In the meconium of calves, the difference in microbial functional hosts is the most remarkable between the EPG and PPG and is also highly matched with the composition of the dominant species in each group, suggesting that the metabolism of the microbiome was mainly manipulated by the dominant bacteria, which might further drive the corresponding functional differentiation [40].

We further evaluated the enrichment of maternal functional genes and their transmission to offspring. As expected, the genes related to starch and cellulose were enriched in the rumen of the EPG, suggesting the ability of the rumen microbiome to efficiently decompose the total mixed ration for the primary material basis of high milk production [17]. For lipid metabolism, we identified genes involved in the synthesis of propanoate, acetate, and long-chain fatty acids. As a precursor of glycogen, propanoate is involved in maintaining the balance of glucose metabolism in the host and is the main source of lactose in milk [41]. Acetate is the most important energy carrier in dairy cows and participates in the synthesis of milk fat [42]. These enriched genes accelerate the metabolic cycle of the rumen microbiome to meet the nutritional requirements of cows for high milk production. In contrast, the synthesis of butanoate and methane was blocked by the related decreased genes. Although butanoate is the most important and direct energy source for rumen epithelial development [43], compared with young ruminants, the rumen morphology of adults is mature and stable [44], which suggests that the niche of butanoate-producing bacteria is compressed and replaced by other nutrient decomposers for the high yield of adult hosts. Additionally, a decline of the methane pathway in the rumen might be a potential sign of high-yield dairy cows because it means that more carbon and hydrogen are precipitated and converted into animal protein in the host rather than being emitted as gas [39]. In the meconium of newborn calves in the EPG, the dominant microbial genes shared with the rumen were mainly enriched in the decomposition of starch, lactose, cellulose, N-glycan biosynthesis, and glycosphingolipid biosynthesis. Liquid feed, including colostrum and milk, is the only food source for newborn calves in early life, and lactose is the most abundant sugar in them [45]. The enriched lactose degradation pathway in the calf gut microbiome can decompose and utilize lactose more efficiently, which is beneficial for calf growth. N-glycan biosynthesis is an important post-translational modification of all immunoglobulins [46], and considering the abundance of immunoglobulin G (IgG) in colostrum [47], glycosylation appears to help it adhere to the intestinal tract and be absorbed. Moreover, glycosphingolipids can be synthesized by gut microbes [48] and have the ability to promote the differentiation of cerebral neuronal cells [49], which might be beneficial for the brain development of calves. Taken together, functional genes enriched in the rumen microbiome of high-yield cows may be transmitted vertically to the gut of calves to maintain their functional dominance.

Although previous studies have reported that breastfeeding might be the main way to transmit resistance to offspring [50], this study showed that before feeding colostrum, the resistance diversity in the meconium of calves was already similar to that of the maternal rumen and feces, and the abundance of ARGs showed an increasing trend, which might be attributed to meconium

resistance originating from multiple maternal sites rather than one [5]. In addition, compared to the PPG, the EPG cows shared more ARG members with their calves, and the total ARG abundance in the rumen and meconium was lower, suggesting that the features of transmission in the cow-calf resistome were regulated by individual physiological states. Moreover, co-occurrence networks of ARG and MGE revealed the possibility of potential transfer of ARGs between mother and offspring via horizontal gene transfer (HGT) [51], especially between the rumen and meconium with the most frequent interactions in the network. According to the above results and discussions, the rumen microbiome was the main maternal source of early gut colonizers of newborn calves. Therefore, the rumen microbial hosts carrying related ARGs and MGEs would directly enter the gut of calves. In addition, compared with the feces, the more frequent interactions of MGEs and ARGs in rumen, meconium, and between the two also indicated that rumen-derived ARGs showed a higher HGT potential, and promoted the secondary diffusion of its resistome in the gut of calves. These evidences implied that the rumen might be the main source of gut ARGs in newborn calves.

According to the 5-site serum metabolomics, we identified core metabolites transmitted from dairy cows to newborn calves. A total of 500 core metabolites were detected at all five sites in both groups, indicating that the essential nutrients newborn calves received from their dams were stable, even if they were connected to different maternal physiological statuses. However, we observed a significant difference in the abundance of core metabolites influenced by maternal characteristics, which is consistent with human research [9]. In both rumen and meconium, pathways related to amino acid metabolism were enriched in most metabolites. Particularly in the meconium, more differential pathways were annotated between the EPG and PPG, implying that the calves further expanded the differences and characteristics of the maternal metabolic profile. For example, tryptophan metabolism is co-enriched in the rumen and meconium. In dairy cows, tryptophan, the precursor of indole acetic acid, promotes the absorption of VFAs by the rumen epithelium [52]. For calves or infants, tryptophan has the ability to protect nerve tissue and improve the antioxidant capacity of the gut [53, 54], which means that the continuation of metabolic characteristics from mother to offspring might perform different functions based on the growth stage of the host. Additionally, although this study confirmed the specific inheritance of calf meconium from the maternal microbiome, the mode and efficiency of transmission of core microbes and metabolites from cow to calf in the complex interaction process still need to be further explored.

Conclusion

To our knowledge, this is the first study to use enterotype analysis and combination methods to screen a population of dairy cows with excellent production traits, thereby emphasizing the collaborative regulatory role of the microbiome in the rumen and hindgut of dairy cows. Based on this, the application of multi-omics further revealed the transmission characteristics of the microbiome and metabolic profile of dairy cows with different physiological phenotypes to their newborn offspring, in which the dominant bacteria, metabolic function, and resistome could be inherited by calves and regulated by maternal features. Our study demonstrates the potential feasibility of dairy cows as a model for investigating the vertical transmission of the microbiome and reveals the characteristics of microbial taxa, metabolic function, and resistome characteristics of maternal and neonatal microbiomes.

Methods

Animals, experimental design, and sampling

A total of 308 transitional dairy cows and their newborn calves were enrolled in this study, which was conducted at the Gansu Tianmu Farm (Jin Chang, Gansu Province, China). We collected rumen fluid, fecal, and serum samples from each cow on the day of delivery, as well as the meconium and serum of the corresponding newborn calves. The rumen fluid was collected using a 2.6-m flexible esophageal tube (SciTech Co., Ltd., Wuhan, Hubei, China) from the cows and the first 10 mL of rumen fluid was discarded to avoid saliva contamination. Then the rumen fluid was filtered through 4 layers of cheesecloth and packed in 5 mL sterilized storage tubes. The samples of feces and meconium were collected with sterilized gloves and packed in 5 mL sterilized storage tubes. The blood samples were collected from cows and calves through the tail vein and jugular vein, respectively, and stored in 10 mL evacuated tubes. Then the blood samples were further centrifuged at 3,500×*g* for 15 min at 4 ℃ to collect the serum into the 1.5 mL sterile frozen storage tubes. All the samples were stored in a − 80 °C refrigerator for the subsequent analysis. In addition, we also backdated and collected the production data of these cows during pregnancy and recorded their individual characteristics, including body weight, parity, gestation day, and body condition score.

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

We used a DNeasy PowerLyzer PowerSoil Kit (Qiagen, Germantown, MD, USA) to extract microbial DNA from the rumen, fecal, and meconium samples. The quality of

the total DNA preparation was checked using a Thermo NanoDrop 2000 UV microphotometer and 1% agarose gel electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene was amplified using the primer pair 338F (5′-ACTCCTACGG GAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) in an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). An AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to extract and purify PCR products using 2% agarose gels, according to the manufacturer's instructions. Products were quantified using a Quantus fluorometer (Promega, Madison, WI, USA). An Illumina MiSeq PE250 platform (Illumina, San Diego, CA, USA) was used to sequence amplicon libraries.

Raw FASTQ files were demultiplexed using an in-house Perl script, quality-filtered using fastp version 0.19.6, and merged using FLASH version 1.2.11 [55]. We selected DADA2 to denoise the optimized sequences. Taxonomic assignment of amplicon sequence variants was conducted in accordance with the naive Bayes consensus taxonomy classifier implemented in Qiime2 and the SILVA 16S rRNA database (v138) [56].

Metagenomic sequencing andbioinformatics analysis

The extracted DNA was fragmented to an average size of approximately 400 bp using a Covaris M220 ultrasonicator (Gene Company Limited, Shanghai, China) to construct a paired-end library using NEXTFLEX Rapid DNA-Seq (Bioo Scientific, Austin, TX, USA). Paired-end sequencing was conducted on an Illumina Novaseq 6000 (Illumina) using a NovaSeq 6000 S4 Reagent Kit according to the manufacturer's instructions. Raw sequencing reads were trimmed of adaptors and low-quality reads (length < 50 bp, quality value < 20, or presence of N bases) were removed using fastp (https://github.com/OpenGene/fastp, version 0.20.0). The clean reads were aligned to the *Bos taurus* genome (GCA_002263795.2) [11], and any hits associated with the reads or their mated reads were removed. MEGAHIT (https://github.com/voutcn/megahit, version 1.1.2) was used to assemble the quality-filtered data. The contigs (length ≥ 300 bp) were selected as the final assembly result. Prodigal (https://github.com/hyattpd/Prodigal, version 2.6.3) was used to predict the open reading frames (ORFs) from each assembled contig and the ORFs ≥ 100 bp in length were also retrieved. A non-redundant gene catalog with 90% sequence identity and 90% coverage was constructed using CD-HIT (http://weizhongli-lab.org/cd-hit/, version 4.7). Gene abundance for a specific sample was estimated at 95% identity using SOAPaligner (https://github.com/ShujiaHuang/SOAPaligner, version soap2.21release). DIAMOND, with an e-value cutoff of 1e − 5 (http://ab.inf.uni-tuebingen.de/software/diamond/, version 2.0.11), was used to obtain the best-hit taxonomy of non-redundant genes by aligning them against the NCBI non-redundant database. The KEGG functional annotation of non-redundant genes was similarly performed [57].

Metabolite extraction, quality control, and analysis

Each 50-mg ruminal, fecal, and meconial sample was added to a 2-mL centrifuge tube, followed by a 6-mm diameter grinding bead. All the samples were ground with a Wonbio-96c frozen tissue grinder (Shanghai Wanbo Biotechnology, Shanghai, China) for 6 min (− 10 °C, 50 Hz) and extracted for 30 min (5 °C, 40 kHz) using low-temperature ultrasonication. The samples were stored at − 20 °C for 30 min, centrifuged for 15 min (4 °C, 13,000 × *g*), and the supernatant was collected for the next step of analysis. Quality control samples comprising a mixture of equal volumes of all samples were prepared to determine the stability of the analysis.

Liquid chromatography-tandem mass spectrometry analysis of samples was performed using a Thermo UHPLC-Q Exactive HF-X system equipped with an ACQUITY HSS T3 column (100 mm length × 2.1 m m inner diameter; 1.8 μm particle size; Waters Corp., Milford, MA, USA). In both positive and negative ion modes, the metabolites eluted from the column were identified using a TripleTOF 5600 Plus high-resolution tandem mass spectrometer (SCIEX, Warrington, UK). The acquired data were exported into the mzXML format using the XCMS software [58]. MetOrigin (http://metorigin.met-bioinformatics.cn/app/metorigin) [19] was used to analyze the traceability and enrichment of metabolites. Using the MetOrigin online server, we selected the Simple MetOrigin Analysis (SMOA) mode for our data analysis. The SMOA mode identified the origins of metabolites based on seven well-known metabolite databases. After loading the dataset, metabolic pathway enrichment analysis was performed. A bar plot was created to summarize the total number of metabolites from the host, microbiota, both host and microbiota and other sources.

Statistical analysis

A one-way ANOVA analysis of variance in SPSS 19.0 (SPSS Inc., Chicago, IL, USA) was used to compare the differences in production performance and individual characteristics. Alpha diversity (Shannon and Chao1 indices), microbiota, and genes between the two groups were compared using a two-tailed Wilcoxon signed-rank test with FDR adjustment, and only bacteria or genes with significant differences ($P < 0.05$) were defined as "enriched bacteria" or "differential gene". Beta diversity based on Bray–Curtis distances were calculated

and tested using an analysis of similarity. The diversity outputs were visualized using the "ggplot2" R package (version 3.6.0). LEfSe (http://galaxy.biobakery.org/), an analytical tool for discovering and interpreting biomarkers of high-dimensional data, was used to identify signature microbiota. $P < 0.05$, and LDA score > 3 were used as criteria for judging the significant effect size.

Previous studies in humans [18] and dairy cows [59–61] based on enterotype analysis have proved that there are subgroups of gut microbiota dominated by different characteristic microbes. Therefore, enterotype analysis was performed using the "cluster" R package (version 3.6.0). Based on the Jensen-Shannon distance between microbial samples, samples were clustered according to the partition algorithm around the central point (unsupervised clustering methods) and the optimal number of clusters was calculated using the Calinski Harabasz index.

Spearman's analysis was performed to calculate the correlations between ARGs and MGEs using the psych R package, and the related interaction networks were visualized using Cytoscape (https://cytoscape.org/). Only significant coefficients ($P < 0.05$, $|r| > 0.5$) are shown in the networks.

The schematic of the workflow was drawn using https://app.biorender.com/user/signin.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-01943-5.

Additional file 1: Figure S1. The microbial community and composition of rumen and feces. A Principal coordinate analysis of the two rumen clusters. B Principal coordinate analysis of the two gut clusters. C Dominant genera across all rumen and gut samples. Figure S2. The main bacteria in the rumen and gut of cows. A The main bacteria in the rumen. B The main bacteria in the gut. The bacteria in red were identified as the shared dominant bacteria in the both group (the relative abundance in both groups > 5%). Figure S3. The microbial association between meconium, rumen and feces. A Principal coordinate analysis of microbiome of rumen and feces in the EPG and PPG. B Wayne diagram showing the microbial intersection of the rumen, feces, and meconium in the EPG and PPG. C The microbial origin of meconium using SourceTracker. FigureS4 Principal coordinate analysis of microbiome of meconium in the EPG and PPG. Figure S5. The microbial α- diversity of rumen, feces and meconium between the EPG and PPG. A The comparison of Chao1 index between the groups. B The comparison of Shannon index between the groups. RF = rumen fluid; FE = feces; ME = meconium. Figure S6. Relative abundances of bacterial communities at the species level. A Relative abundance of bacterial communities at the species level in the rumen of EPG and PPG. B Relative abundances of bacterial communities at the species level in the feces of EPG and PPG. C Relative abundances of bacterial communities at the species level in the meconium of EPG and PPG. Figure S7. The difference of KEGG metabolic function attached to meconium microbiome between the EPG and PPG. A Significantly different KEGG pathways related to metabolism of meconium microbiota in the EPG and PPG. B Heatmap depicting the difference of microbial hosts of metabolic functions at the species level in the meconium. Figure S8. The abundance of MGE class in the rumen, feces and meconium between the EPG and PPG. A The total abundance of MGEs. B The abundance of integrall. C The abundance of ICEberg. D The abundance of ISFinder. E The abundance of Plasmid. TPM = Transcripts per million. Figure S9. Volcano map of metabolites identified by the metabolome of rumen, feces and meconium between the EPG and PPG. A The identification of signature metabolites in the rumen. B The identification of signature metabolites in the feces. C The identification of signature metabolites in the meconium. Figure S10. The identification of metabolites of rumen, feces and meconium from different sources. A The source of rumen metabolites. B The source of feces metabolites. C The source of meconium metabolites. Figure S11. The enrichment of fecal core metabolites. A Metabolic pathway enrichment analysis of differential core metabolites in the feces. B The pathway enrichment analysis of metabolites and its association network in the feces. The major metabolic pathways are marked in orange nodes and the nodes of signature metabolites are marked in red (enriched in the EPG) or blue (enriched in the PPG) in the association network. Table S1. Comparison of phenotypes of cows between the RUE1 and RUE2. Table S2. Comparison of phenotypes of cows between the GUE1 and GUE2. Table S3. Comparison of phenotypes of cows between the EPG and PPG.

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Authors' contributions
Z.C. served as principal investigators. M.L., W.W., and S.L. designed the experiments. Y. Z., and D.G. analyzed metagenomic data, 16S rRNA gene sequencing and drafted the manuscript. S.L., W. J., Y. X., T. C., J. X., S. L., G. H., X. Z., S. L., S. Z., J. W. and Y. H. contributed to collection of samples. All authors discussed the results, critically reviewed the text, and approved the final manuscript.

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Data availability
The accession for the 16s sequencing and metagenomic data in this study are in the NCBI Sequence Read Archive: # PRJNA1078885.

Declarations

Ethics approval and consent to participate
Animal experiments were performed in accordance with the Regulations of the Administration of Laboratory Animals (2017 Revision) promulgated by Decree No. 676 of the State Council, China. The animal care protocol was approved by the Animal Care and Use Committee of China Agricultural University (Protocol Number: AW10803202-3–2).

Consent for publication
Not applicable.

Competing interest
The authors declare no competing interests.

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