Changes in host liver transcriptome and rumen microbiota community in fattening goats fed different type of forages

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Research Article

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Abstract

Background: To explore the effects of different forages on rumen flora and expression of genes related to liver metabolism in fattening goats, Boer crossbred male goats were fed three experiment diets lasts for 97 days, including an adaptation period of seven days. The diets were composed of same commercial concentrate, but supplemented with different forages (Hemarthria altissima (HA), Pennisetum sinese (PS), or forage maize (FG)). The liver transcriptome changes along with its correlation with the rumen microbiome changes were investigated in these animals.

Results: Transcriptome results revealed a total of 73 differentially expressed genes (DEGs) between FG and HA groups, and biological pathways enriched by these DEGs included circadian regulation of gene expression, transforming growth factor beta receptor signaling pathway, lipid metabolic process, and multiple KEGG pathways related to energy metabolism, such as AMPK signaling pathway, Choline metabolism in cancer, Insulin resistance, cAMP signaling pathway, and Insulin signaling pathway. Furthermore, our 16S rRNA gene sequencing results revealed a remarkable difference in rumen microbiota composition due to different dietary treatment, and the correlations between the relative abundances of bacteria at the genus level and the expression levels of DEGs were analyzed. By which, nine bacteria genera exhibited at least a strong correlation to at least one of these DEGs. Among these bacteria genera, the *Muribaculaceae*was reported with involvement in fat metabolism, which exhibited the highest number of associations overall with 18 strong correlations with DEGs, implying the direct relationship between rumen *Muribaculaceae*abundance and liver gene expression changes.

Conclusions: Our study provides insight into the physiological axis linking the rumen microbiome and liver genes to forage utilization in goats, which may facilitate future studying the underlying molecular mechanism of how forages lead to the improvement of the nutritional value through the microbiota-gut-liver axis in ruminants.

Background

The rumen of ruminants plays a fundamental role in digestion and metabolism. It harbors trillions of microbes, which utilize dietary- and host-derived molecules generating a diverse group of metabolites. Diet has a large economic impact on raising processes, and the composition and source of diet dramatically impacts the profile of rumen microbial community and animal health[1]. For example, legumes and grasses mixed pastures are associated with rumen microbiota composition and enhancing animal growth performance in grazing ruminants as compared to monoculture pastures[2, 3]. Furthermore, the rumen microbiota was characterized by a dynamic and complex network of interactions with the host, and the crosstalk between rumen microbiota and host depends on host genetics and deterministic environmental factors such as diet, and stochastic factors such as antibiotic exposure[4].

The liver plays a central role due to its wide range of functions in body homeostasis, metabolism of glucose, lipids, proteins, as well as regulation and detoxification of ion gradients[5]. Any changes in

dietary nutrient will affect rumen microbial population and its function[6], and emerging evidence indicates that the gut microbiota could affect liver function[7]. According to Wang et al[8], a long-term high-energy diet increases damage of rumen microbiome and promotes hepatic inflammatory response and cytokine/chemokine-mediated signaling pathways. To date, the gut-liver axis is commonly regarded as the most important bidirectional communication that enables the gut microbiota to strongly affect animals' feeding behaviors and metabolism[7, 8]. Thus, it is reasonable to hypothesize that the liver and rumen microbiota will responsive to forage types due to their central role in maintaining the overall metabolism of organism.

Nowadays, multi-omics approach has been widely applied in complex traits and disease study to explore their potential molecular mechanisms[9, 10]. The transcriptome could identify differentially expressed genes and provide comprehensive insight into the molecular mechanisms at the transcriptional level. Recently, RNA-seq has been used in whole-genome analysis of liver tissue of pig[11], rabbit[12], sheep[13, 14], cow[15], and goat[16] at the transcriptional level, and many genes may regulate meat quality has been identified. As a culture independent technology, 16S rRNA based gene sequencing technique plays crucial function in characterizing rumen microbial composition and diversity difference after feeding different types of forages and has made possible in systematically investigating the relationship between rumen microbiota and meat quality traits[17]. Moreover, the integration transcriptomic and metagenomics can help evaluate the gastrointestinal microbiota and understand the metabolic status in the liver[8]. We have previously estimated the role of rumen microbes in goats by evaluating the diversity and relative abundance of bacteria related to meat quality, and found goats fed forage maize can improve their meat nutritional value[17]. Besides rumen microbiota composition, regulation of nutrition metabolism in liver affects animals' performance[13]. Therefore, it is critical to study the candidate genes and genomic networks for understanding the metabolic status in the liver of goats fed forage maize. In addition, we aimed to identify how forage maize leads to the improvement of the nutritional value through the microbiota-gut-liver axis.

Results

RNA sequencing and mapping of reads

We constructed thirteen cDNA libraries, and a total of 284,825,560 raw reads were generated with a length of 150 bases. After filtering adaptor sequences and low-quality reads, a total of 284,453,997 clean reads were remained. More than 73.43% of the clean reads were successfully mapped to the goat genome using HISAT2 software. After filtering genes with less than one raw count read in average, expression of 15,544 annotated genes (covering 61.82% of 25144 gene set) was identified in at least one animal. According to all the gene expression levels, all libraries were clustered by using principal component analysis (PCA). The first two principle components accounted for 46% of total variance (Fig. 1A). Furthermore, the read count data were used to estimate the distribution pattern, and our results showed that the read count distribution for the expressed genes were similar among the three groups (Fig. 1B).

Identification of differentially expressed genes

Each experimental group was compared against one another to check the differentially expressed genes (DEGs) based on read count values of each gene. Between HA and PS groups, and FG and PS groups, only four and three genes were detected to be differentially expressed, respectively (Table S1). Besides, we identified a total of 73 DEGs of which 60 were upregulated and 13 were downregulated in FG compared to HA group (Table S1; Fig. 2A). A clear separation between the FG and HA goats was observed in the 73 DEGs (Fig. 2B). In particular, we were interested in comparing FG group to the others because FG group could induce an improvement of meat nutritional value[17].

GO and KEGG pathway analysis of differentially expressed genes

To investigate the functional association of the DEGs identified from FG and HA groups, we determined their functional category by querying Gene ontology (GO) database. There were 86 GO terms with Padj < 0.05 (Table S2), of which these categories containing at least three genes in biological process, cellular component, and molecular function were shown in Fig. 3. The enrichment results revealed that these genes are related to many biological processes that are important for the gene transcription and host lipid metabolism, such as negative/positive regulation of transcription by RNA polymerase II, circadian regulation of gene expression, transforming growth factor beta receptor signaling pathway, regulation of lipid metabolic process, and negative regulation of apoptotic process. The cellular components associated with these genes include cell division, nucleus, cytosol and dephosphorylation, and the molecular functions corresponding to these genes include lipid metabolic process, protein binding, chromatin binding and ubiquitin protein ligase binding.

Mining KEGG database using the 73 DEGs retrieved 19 significant pathways, and the most represented pathways includes AMPK signaling pathway, Endocrine resistance, Choline metabolism in cancer, Insulin resistance, Non-alcoholic fatty liver disease (NAFLD), cAMP signaling pathway, and Insulin signaling pathway (Fig. 4).

Correlations between the rumen microbiome and liver transcriptome

The rumen liquid samples were analyzed for phylum and genus level differences through 16S rRNA gene sequencing to explore the effects of forage type on the rumen and ruminants in previous laboratory studies, by which we found that the forage changes can alter the rumen microbiota[17]. Herein, correlations between the relative abundances of rumen bacteria and the expression levels of DEGs were investigated by spearman correlation analysis (Fig. 5). There were nine bacteria genera that exhibited at least a strong correlation ($|r| > 0.6$; six positive and 37 negative) to at least one of the 27 DEGs. The *Muribaculaceae* exhibited the highest number of associations overall with 18 strong correlations with

DEGs (one positive and 17 negative), and a significant number of associations were observed with other bacteria genera, including *Bacteroides* (n = 10), dgA-11_gut_group (n = 3), and *Alistipes* (n = 3).

Discussion

Roughage plays a vital role in ruminant and other herbivore diets, which affects the performance of animals and the quality of products. In ruminants, roughage factors such as fibre content, particle size, roughage source, processing degree can impact methane emissions and feed efficiency[18]. The results of numerous studies confirmed that the relationships between forage and production traits and healthy issues of ruminants such as milk protein nutrition[19], meat quality[20], growth performance and nutrient digestibility[21], liver abscess[18]. Even though the utilization of forage-based diets in ruminant have been explored for hundreds of years, the understanding of metabolic mechanisms under different forage sources is still limited. For goats, the rumen and liver are two extremely important organs involved in metabolism and production. Our previous study found that after 90 days of feeding with the Hemarthria altissima, Pennisetum sinese or forage maize, the growth performance, carcass trait, meat quality, and rumen microbiota of goats were changed[17], but simultaneous changes in liver metabolism under these forage resources are not well known. We designed the experiment assuming that the forage type plays a key role in changing transcriptome pattern of liver. Indeed, the effect of different forage resources on liver metabolism have been investigated by using RNA-seq and bioinformatics analyses in cows[22].

In the current study, only four and three genes were detected to be differentially expressed between goats in HA and PS groups, and FG and PS groups, respectively. The liver gene expression of goats was changed significantly between FG and HA groups with a total of 73 DEGs. We further conducted the GO enrichment and pathway analyses of the DEGs screened from HA and PS groups, and found that these genes are significantly enriched in signaling pathways that regulate energy metabolism, including lipid metabolic process, regulation of lipid metabolic process, transforming growth factor beta receptor signaling pathway, fat cell differentiation, response to glucagon, response to cAMP, cAMP signaling pathway, AMPK signaling pathway, and cholesterol metabolism. The liver is a special organ to regulate metabolism and which was sensitive to the nutrients it takes in[5]. As an example, a recent study suggested that roughage source and its factors are complex to rumen kinetics and liver metabolic status[18]. In addition, the emerging axis of the host physiology and the gut microbiome provides therapeutic targets to prevent and treat liver metabolic diseases and enhance host health[23]. Further, some of the genetic variation in efficiency of nutrient use could be due to differences in the regulation of gene expression[24]. Our study suggests that these genes involved in the energy metabolism are poised to interpret biological adaptions of liver to forage types.

Ruminants and microorganisms are in constant dynamic balance of mutual dependence and restriction, and forage type can change microbes dramatically to adapt it[25]. The gut-liver axis plays crucial roles in nutrition absorption as the liver receives approximately two-thirds of blood from intestine. Consequently, the liver is the first recipient of gut-derived dietary nutrients, thereby acting as the most important metabolic organ than others[7]. High grain diets always induced ruminal microbiota change, and host

immunity could act on gut microbiome diversity[26]. As in our previous study, feeding different type of forages leads to marked alterations in rumen microbiota. In the present study, the results showed that nine bacteria genera had significant correlation with the 27 DEGs. Among these genera, the *Muribaculaceae* exhibited the highest number of associations overall with 18 strong correlations with DEGs. It has been reported that the abundance of *Muribaculaceae* was positively covaries with resistance to fat[27, 28], which may be related to absorption and utilization of fat[29], indicating this genus has played important role in modulating the fat metabolism in goats. Thus, further investigation on microbiota-gut-liver axis may shed light into the responsive mechanism associated with *Muribaculaceae* in goats.

Notably, several limitations of this research that need to be considered. First of all, a relatively small sample size was enrolled to explore the liver transcriptome and rumen microbiome profiles, which are prone to generating false-positive correlations between genes and bacteria genera. Our hierarchical cluster analysis on the DEGs revealed a clear between-group differences and within-group similarities (Fig. 2B), indicating the sequencing results were accurate and reliable. Further experiments are needed to validate these correlations and to examine their roles in forage utilization. Secondly, the rumen fermentation parameters and metabolites in both rumen and liver are not recorded in these animals, as the microbiota-host communication is based on several gut-derived compounds and then translocated into the liver[30]. Therefore, further studies are necessary to elucidate these factors.

Conclusion

This study captured liver transcriptome changes in response to forage type in fatten goats. Importantly, functional confirmation of the DEGs involved in liver pathways related to lipid metabolism and their association with rumen microbes will help the future investigating of forage nutrition utilization. Specifically, the underlying molecular mechanism of *Muribaculaceae* in the microbiota-gut-liver axis may facilitate illustrating the biological process.

Methods

Study design and sample collection

This study is a continuation of previous research, where the rumen microbiota, growth performance, carcass trait, and meat quality of fatten goats were investigated[17]. However, this research focuses on the liver transcriptome changes resultant from the forage types. Briefly, a total of 13 healthy 6-month-old male Boer crossbred goats of the same weight were selected for this study. All the animals were housed in individual wooden pens on a raised slatted floor and received the same concentrate but different type of forages. Animals were divided into three groups of HA (N = 3), PS (N = 5), and FG (N = 5) according to the forages of Hemarthria compressa, Pennisetum sinese, and forage maize, respectively. For each group, the concentrate was offered 3.5kg per day for each, whereas forage and water were both offered *ad libitum*. The goats were fed for 7-day pre-trial period and 90-day positive trial period, and then weighted

and slaughtered according to standard protocol at the end of the experiment. After dissection, the rumen fluid and liver samples were collected and quick-frozen in liquid nitrogen, whereas the carcasses were stored at 4℃ for 24h until meat quality measurements.

RNA extraction, RNA-Seq library construction and sequencing

Collected liver tissues were homogenized in liquid nitrogen into a fine powder. Total RNA was isolated using TRIzol Reagent (TaKaRa, Dalian, China), and DNA was cleaned out using the RNeasy Midi Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols. The quality of extracted RNAs was assessed using Nanodrop 2000C (Thermo Fisher Scientific, Wilmington, DE) and Qubit Fluorometer (Invitrogen, Carlsbad, CA). RNA-seq library preparation was done using an Illumina TruSeq ribo-zero gold kit (Illumina, San Diego, US). Sequencing was conducted on an Illumina NovaSeq 6000 instrument to generate 150 bp paired-end reads.

Reads mapping and differential gene expression analysis

Adaptor sequences removal and low-quality reads filtering were conducted according our previous work[31]. Subsequently, the high-quality reads were aligned to the latest goat (*Capra hircus*) reference genome (*ARS1*) using HISAT2 software (v2.1.0) with default parameters[32]. Read numbers mapped to each gene were counted by featureCounts (v1.5.0-p3)[33]. The differentially expressed gene (DEGs) analysis among different groups was performed by using DESeq2 R package (v3.2.3)[34], for which genes with less than thirteen raw reads was considered as cutoff to exclude genes from further analysis, and only those with both adjusted p-value (padj) < 0.05 and |log2(FoldChange)| > 1 were considered as DEGs. Gene function annotation and pathway analysis were performed using KOBAS sever[35].

Joint analysis of transcriptomic and metagenomic data

The microbial community differences among dietary treatment were investigated in our past study[17]. To access the potential regulatory network of meat quality diversity regarding forage alteration, spearman correlation coefficients were calculated between host liver metabolism and rumen microbial composition. To do this, the DEGs from liver tissue and bacterial taxa at the genus level were included in the analysis through using the Corrplot R package, and the strong correlation was defined with the threshold |r| > 0.6 and p-values < 0.05.

Abbreviations

HA Hemarthria altissima

PS Pennisetum sinese

FG Forage maize

DEGs Differentially expressed genes

GO Gene ontology

Declarations

Supplementary Information

The online version contains supplementary material available at https://xxxxxxxxx

Additional file 1: Table S1. Differentially expressed genes identified in goat liver.

Additional file 2: Table S2. Gene Ontology analysis of differently expressed genes between FG and HA goat liver.

Ethics approval and consent to participate

The authors confirm that all methods were performed in accordance with relevant guidelines and regulations of Chengdu University, China. All animals handing mentioned were reviewed and approved by Institutional Animal Care and Use Committee of Chengdu University (SSXY-600008).

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request. The RNA-seq data generated in this project has been deposited in the Sequence Read Archive (SRA): PRJNA995354, and the 16S rRNA gene sequencing data has been deposited in the SRA of PRJNA906259.

Competing interests

All authors have no conflicts of interest to declare.

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Authors' contributions

Conceived and designed the experiments: ZW and YX. Performed the experiments: ZW, MX, WH, XL, QL, JZ and YC. Analyzed the data: ZW, WW, and DL. Wrote the paper: ZW. Reviewed and edited the manuscript: ZW, MX and YX. All authors read and approved the final version of the manuscript.

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Not applicable.

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Figures

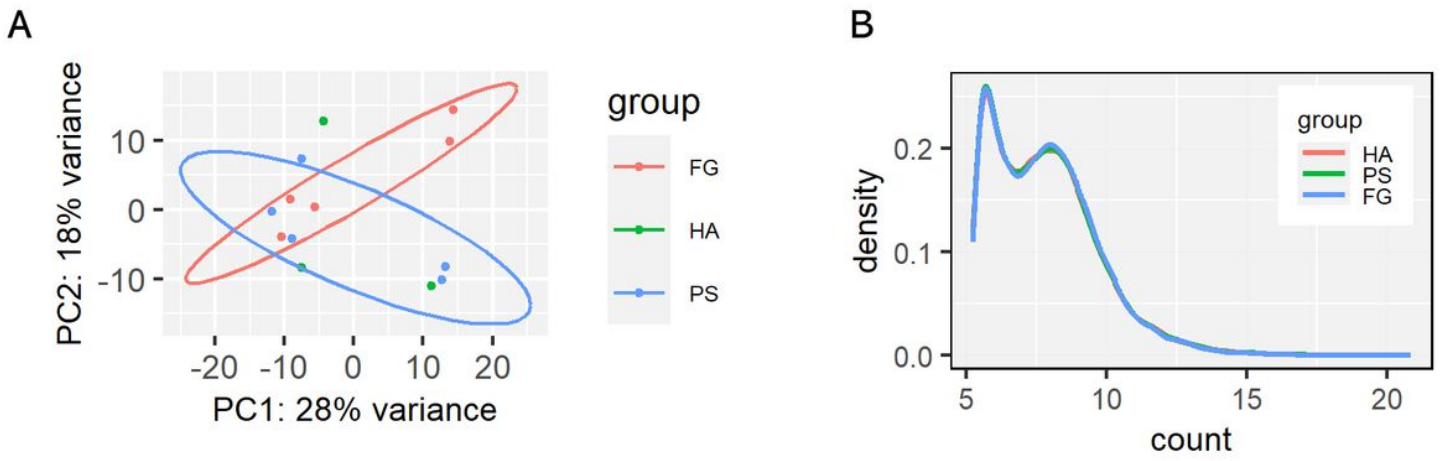

Figure 1

Gene expression patterns of all samples among the three groups. (A) The principal component analysis (PCA) plot of normalized read counts of all samples and (B) the density plot of normalized read counts for each group.

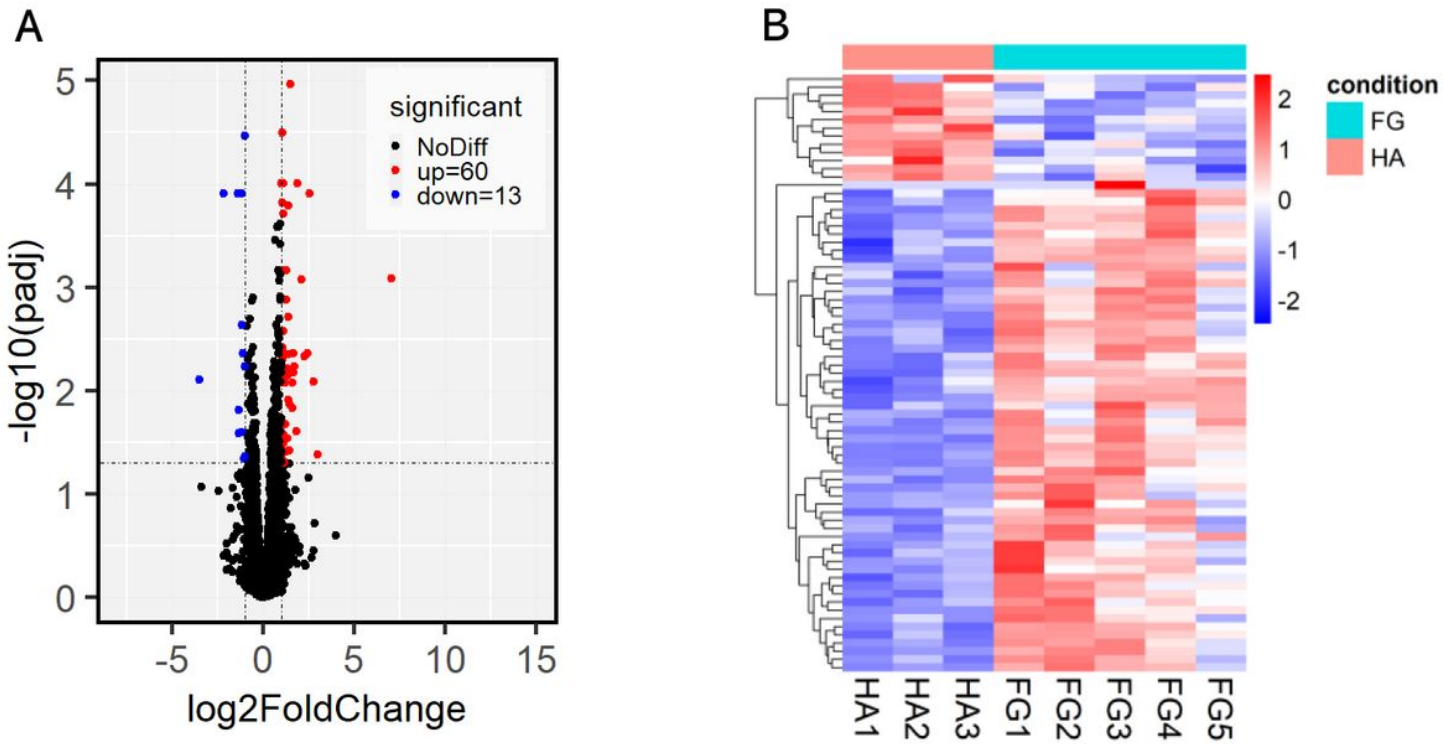

Figure 2

Screening of differential expressed genes between FG and HA goats. (A) volcano plots of differential expressed genes. (B) Hierarchical cluster analysis of differential expressed genes.

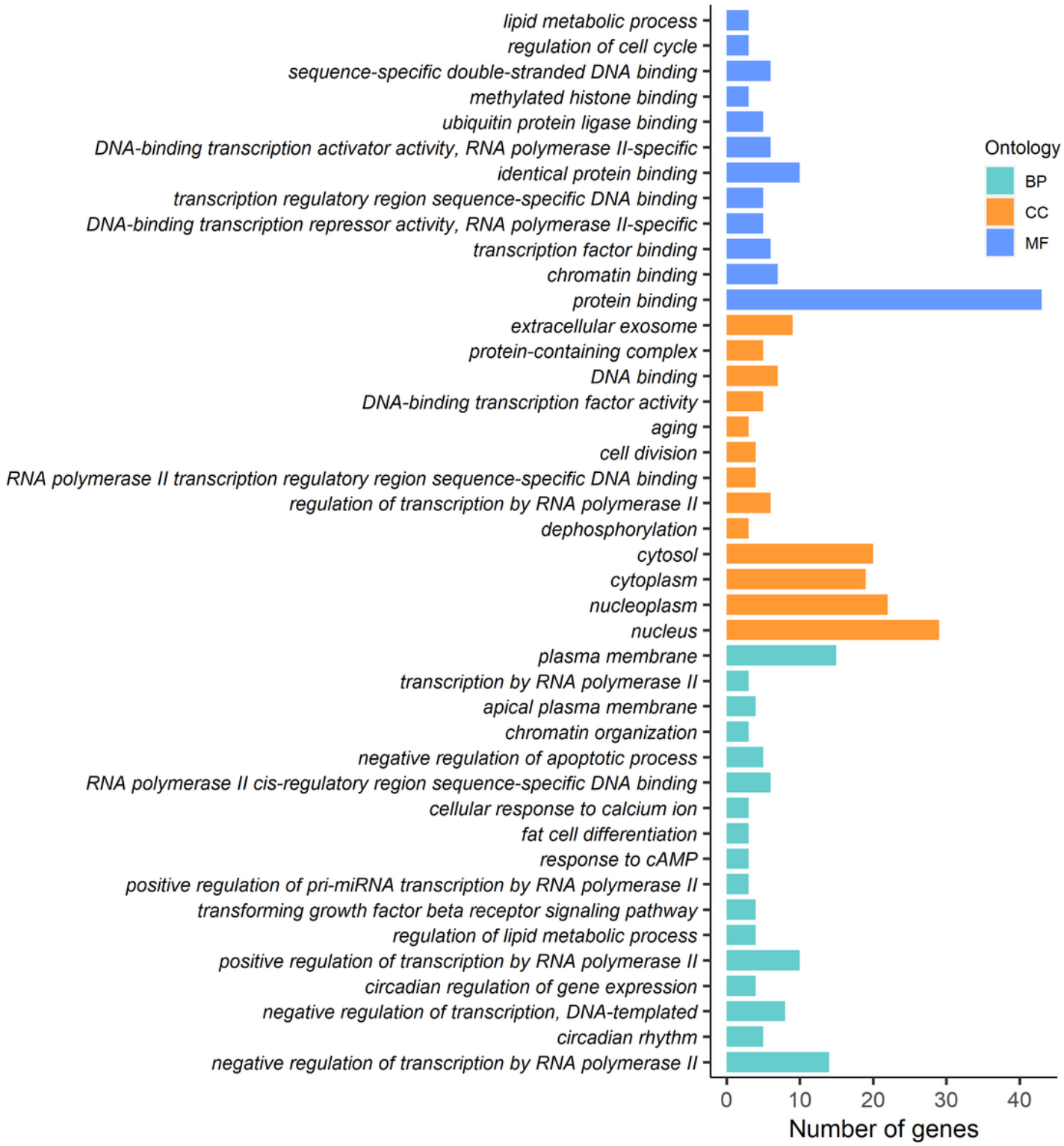

Figure 3

Gene ontology (GO) analysis of differentially expressed genes between the FG and HA goats. The identified 73 differentially expressed genes were classified into three categories: biological process, cellular component and molecular function.

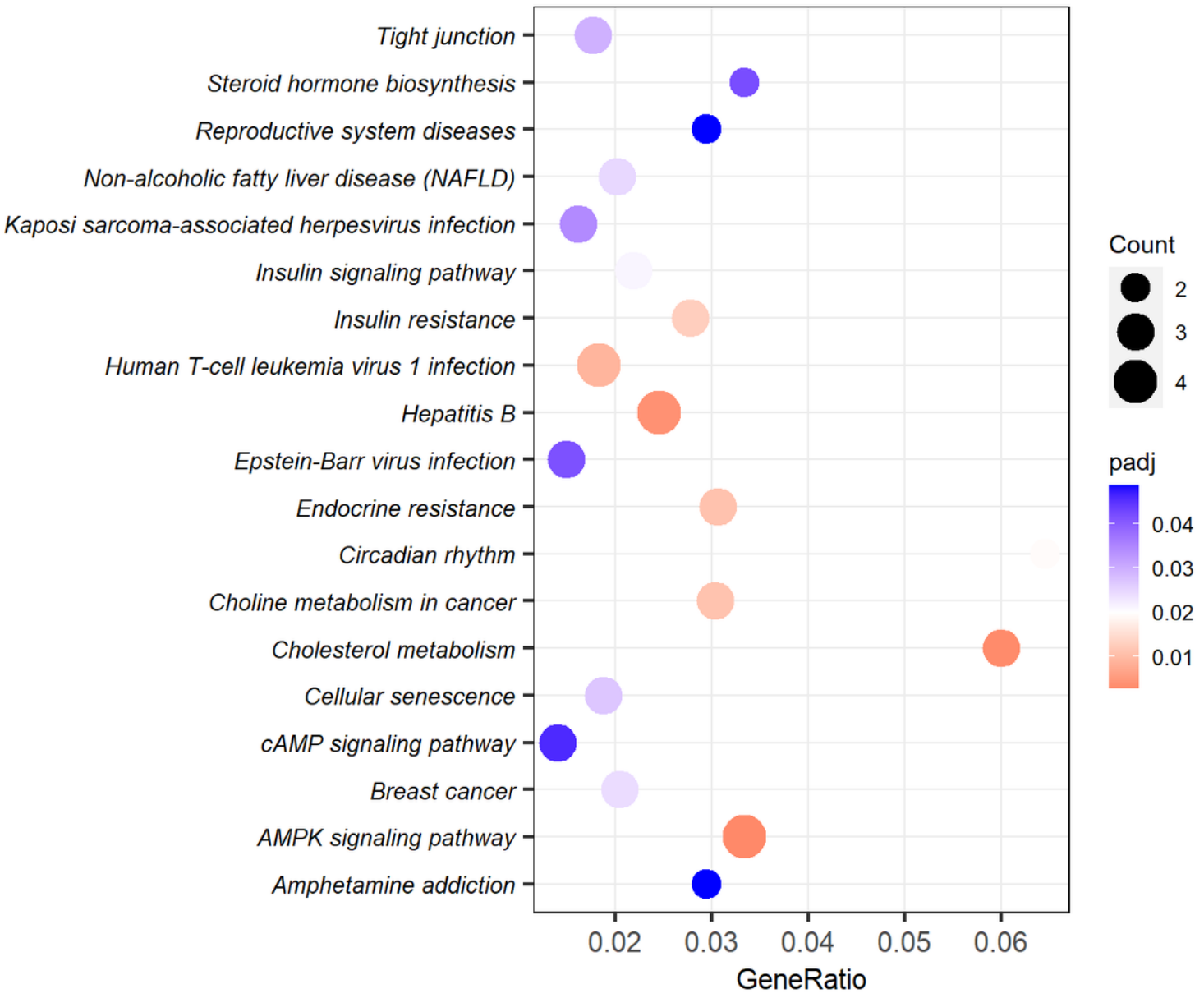

Figure 4

KEGG analysis of differentially expressed genes between the FG and HA goats.

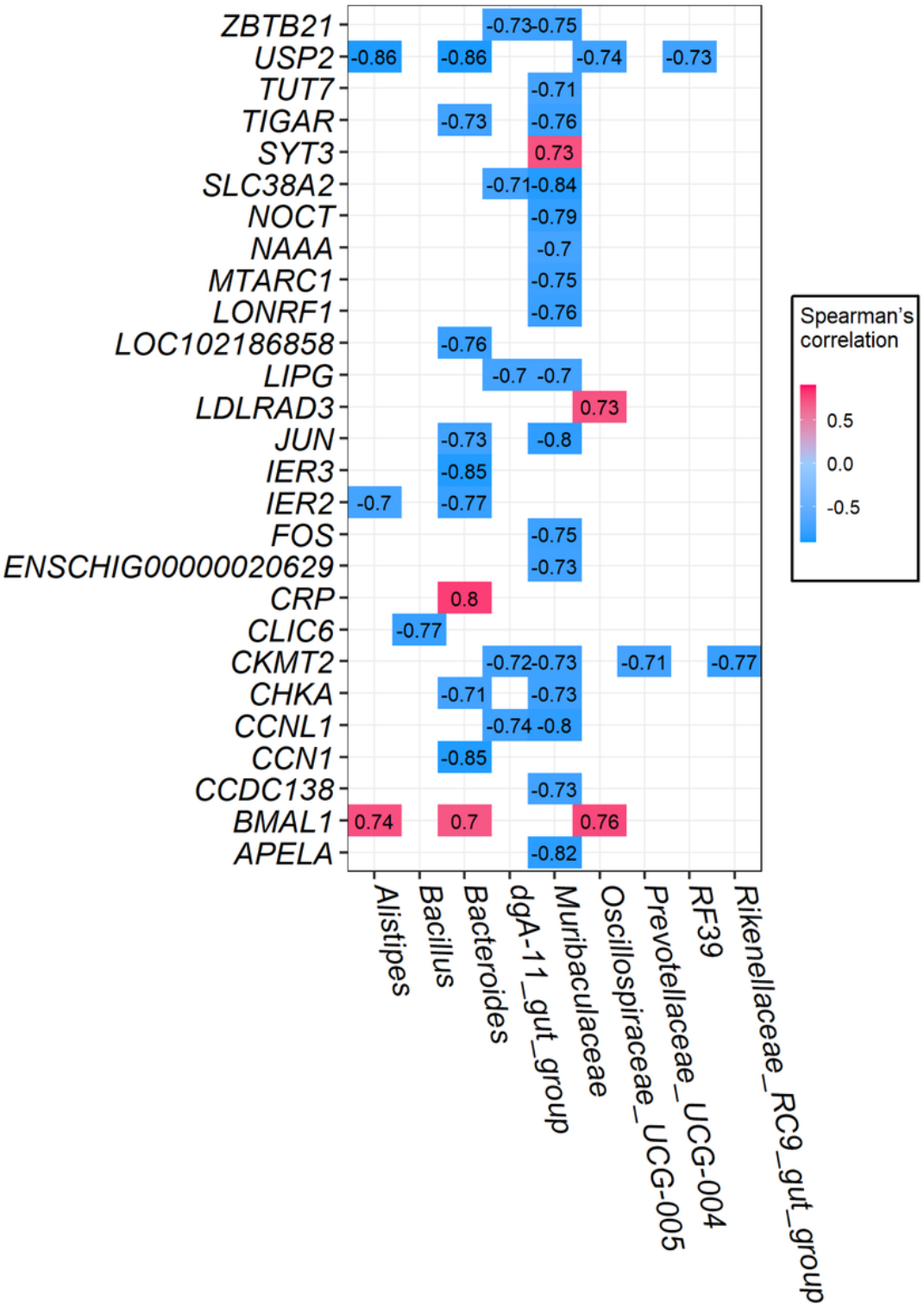

Figure 5

Heatmap of the strong correlated (|r| > 0.6) bacteria and DEGs. Each row in the graph represents a genus, and each column represents a DEG.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.Differentiallyexpressedgenesidentifiedingoatliver.xlsx
- TableS2.GeneOntologyanalysisofdifferentlyexpressedgenesbetweenFGandHAgoatliver.xlsx