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Blood transcriptome pathway analysis of methane production for beef-on-dairy cattle

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Introduction

Methane (CH_4) emissions present significant obstacles to both environmental sustainability and energy efficiency in ruminant including beef cattle that born to the dairy herds. Although numerous approaches, including alterations in feed and the use of additives are under investigation to mitigate these emissions, the genetic selection of animals that produce lower levels of methane offers the potential for enduring and cumulative advantages. Transcriptome analysis represents a crucial advancement in elucidating the networks and mechanisms through which the ruminant genome influences methane emissions.

Materials & Methods

Male beef cattle born in dairy herd (n=11) were fed a diet consisting of high quality grass silage and 5 kg commercial concentrate, of which 1 kg served as bait feed to encourage visits to an Automated Head-Chamber System (GreenFeed, C-Lock Inc.) for enteric gas monitoring. Mean of 30 daily methane measurements, as gram CH4 per day, was calculated for each animal. The feeding schedule was 6 visits per day with 6 drops per visit and 35 seconds intervals. Blood samples were collected from the slaughtered animals into PAXgene Blood RNA tubes (Qiagen) and sent for high-throughput RNA sequencing by Illumina NovaSeq Platform.

The paired-end RNA-seq data (30 million reads coverage) were received and subjected to pre-processing, including quality control (FastQC, V0.11.9) and trimming (Trimmomatic, 0.39). The high-quality read sequences were used for alignment on the Bos taurus reference genome, ARS-UCD1.2 by STAR, 2.7.11b. Samtools 1.10 and FeatureCounts, 2.0.2 were used to index the aligned sequences and create a read count matrix.

The count matrix and the metadata file including methane production per animal were applied as input files for iDEP 2.0 and iDEP.96 and differential gene expression using DESeq2 package in R (v 4.4.0). Methane was in DESeq2 as a continuous trait. iDEP normalized and filtered out the genes if they have no expression in any of the sample or with extremely low expression (counts per million or CPM < 0.5).

Pathway enrichment analysis by iDEP was performed by refined genes and significant pathways were obtained according to false recovery, FDR, rate below 0.05. The top ten pathways, ranked based on the FDR, were presented.

Results

Emissions varied from 233.5 to 332.16 g/day (mean ± SD: 291.18 ± 25.85 g/day). Differential expression analysis identified 1eleven genes with nine annotated gene symbols significantly associated with methane emissions (Padj <0.05), including 4 downregulated (KIAA1211L, OSCP1, IL12B, FREM1) and 5 upregulated (DSCAML1, OSBP2, ACAN, PRSS16, CD1B) genes. The log2 fold change and functions of these genes have been presented in table 1.

After iDEP preprocessing the total number of 15,181 genes passed the filter and 12980 genes were converted to Ensembl gene IDs, and 2201 genes were kept in the data using original IDs. In the presented networks, after pathway enrichment, two pathways (nodes) are connected if

they share 30% (default, adjustable) or more genes. Green and red represent up- and down-regulated pathways. Darker nodes are more significantly enriched gene sets. Bigger nodes represent larger gene sets. Thicker edges represent more overlapped genes.

Table 1. Gene function of differentially expressed genes.

Gene symbol	log2Fold Change	Gene function	FDR
KIAA1211L	-0.62	Miosis, Spermatogenesis, Fertility	1.22×10^-5
OSCP1	-0.32	Plasma membrane transporter of organic solutes, Sulfate importer	1.21×10^-2
IL12B	-0.40	Innate and adaptive immunity, Allergic response	2.68×10^-2
FREM1	-0.53	Extracellular matrix molecule, Cell adhesion	2.06×10^-2
DSCAML1	1.62	Nervous system, Fertility	0.03
OSBP2	0.88	Sexual maturation	0.01
ACAN	1.33	Skeletal development	0.01
PRSS16	2.38	Immune system, Thymus-specific serine protease	0.03
CD1B	1.76	Pathogen response, Immune system	0.03

The top 10 significantly enriched pathways with lowest FDR (adjusted p-value), using KEGG enriched pathways analysis based on k-means clustering with 2000 top genes and 2 clusters in Bos taurus species are presented in Figure 1.

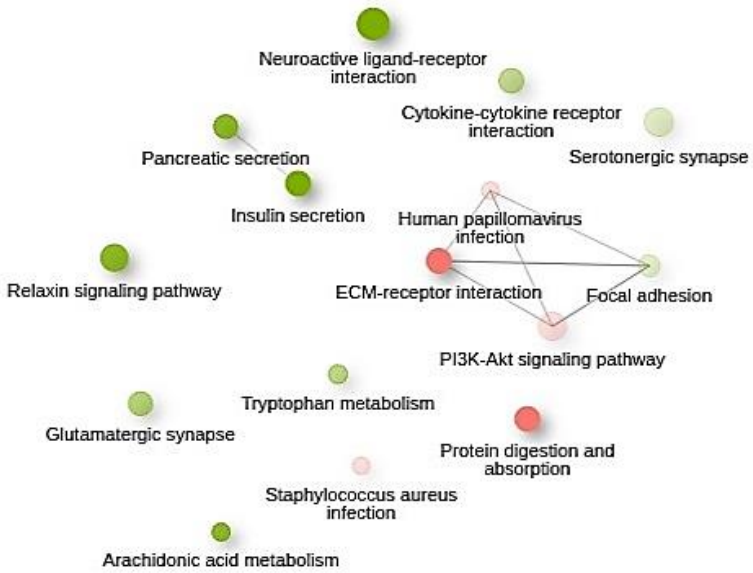

Figure 1. KEGG enriched pathways analysis based on k-means clustering).

GO biological process, GO molecular function and GO cellular component were enriched based on k-means clustering with 2000 top genes and 2 clusters are presented in figure 2, 3 and 4 respectively. The main enriched KEGG pathways were related to the neural signalling and synapses, infection and immune system response and tryptophan metabolism. GO molecular function enrich pathways related to cell membrane transport, cell and extra cellular matrix signalling. The main identified GO biological pathways were immune system, cell adhesion

and extracellular matrix and ion cell membrane transport. Identified GO cellular component were cell membrane ion transport, extracellular matrix and plasma membrane.

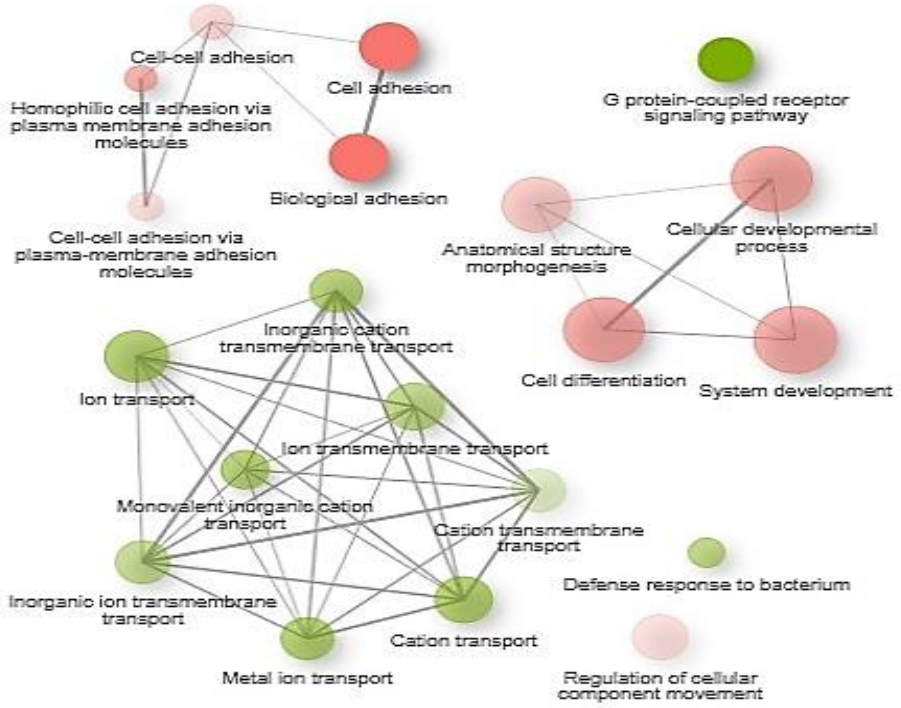

Figure 2. GO biological process enriched pathways based on k-means clustering).

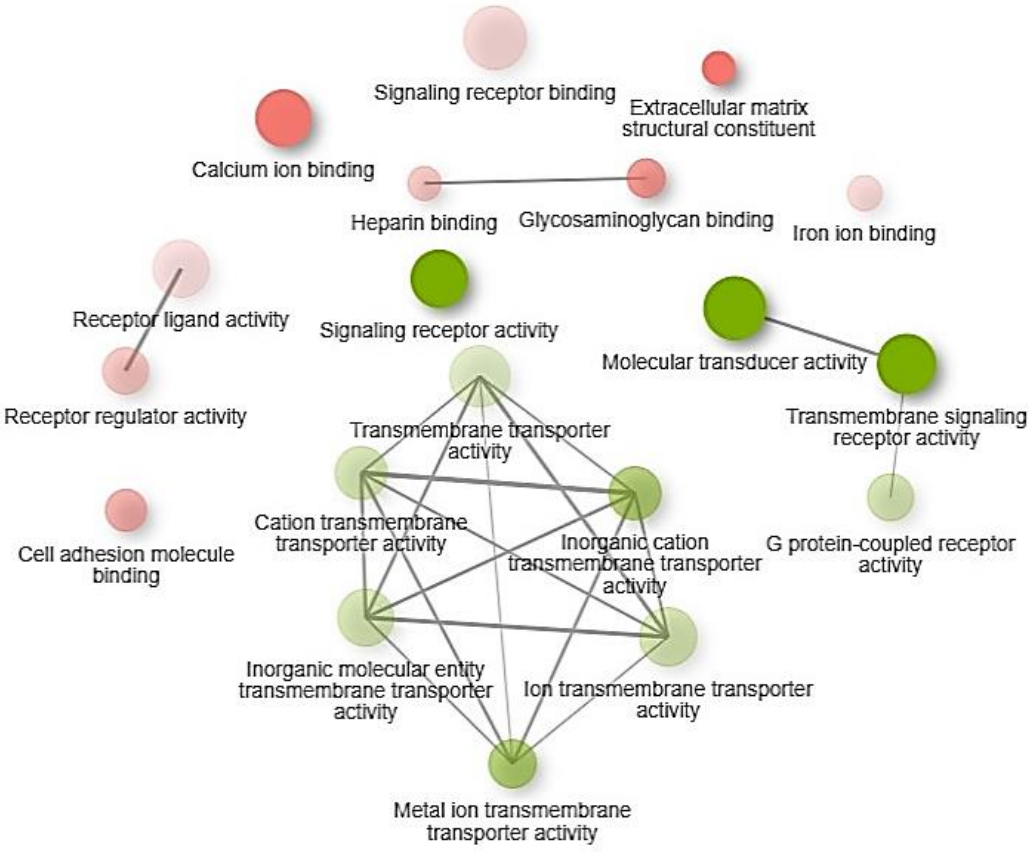

Figure 3. GO molecular function enriched pathways based on k-means clustering.

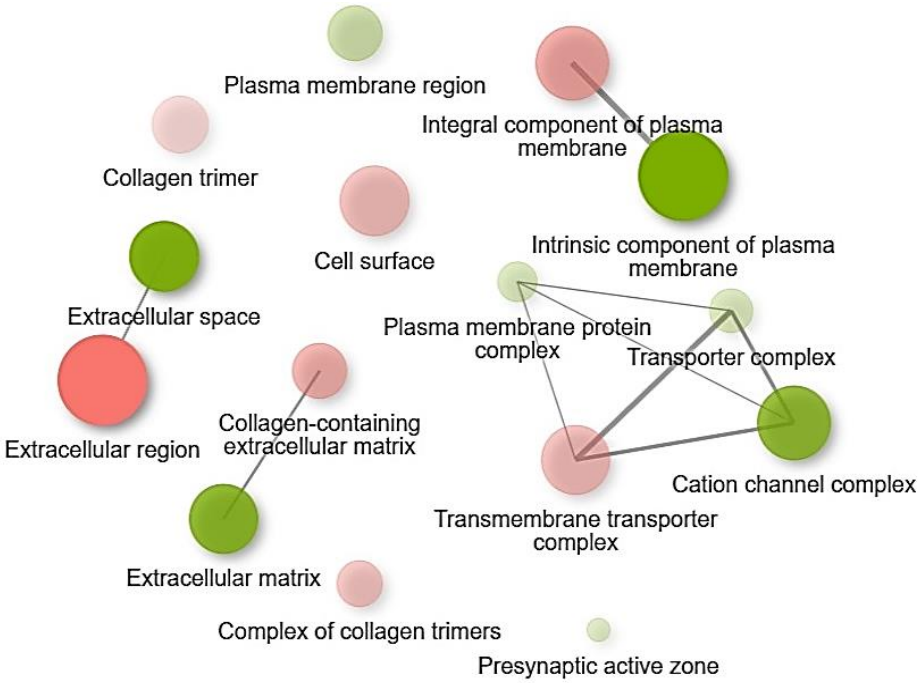

Figure 4. GO cellular component enriched pathways based on k-means clustering.

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Discussion

The molecular and cellular bases of ruminal methane production in dairy beef cattle have been elucidated in the present investigation. High throughput RNA sequencing and RNA-seq data analysis helped to identify nine annotated differentially expressed genes and multiple potential biological systems and pathways related to methane emission in beef cattle. Further investigation of these findings could be valuable for both future applied and fundamental research endeavours. In addition to the cell defence and immune-related, cell-adhesion and extracellular matrix component, cell membrane ion transport pathways applied in nervous system signal transduction, serotonergic synapse and tryptophan metabolism pathways were also enriched. These pathways may exert their effects on neural activity and systemic inflammatory cascade through kynurenine degradation pathway that catabolises tryptophan (Roth, et.al., 2021). Ninety five percent of serotonin is secreted by the digestive system (Bellono et.al., 2017) and this neurotransmitter involved in digestive tract motility, secretion, anti-inflammatory activity, and behaviour (Roth, et.al., 2021; Guzel et.al., 2022). This is in line with advances and discoveries revealing the microbiota-gut-brain axis (Huang et. al., 2025). At the cellular and molecular level, the enriched pathways include extracellular matrix, cell membrane, membrane transportation, synapses and signal transduction.

Conclusion

These results shed light on pathways that may be related to the ruminal methane production and collectively show that this process could be influenced by the rumen microbiome, inflammatory and nervous systems.

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