***Application:*** Methane emissions from ruminants pose significant challenges to environmental sustainability and energy efficiency in sheep farming. This study investigates the blood transcriptome of lambs with varying methane emissions to identify genes linked to methane production, paving the way for the genetic selection of low methane-emitting animals as a cost-effective and sustainable strategy.

***Introduction***: The growing demand for meat drives increased sheep farming, contributing to higher methane emissions and other negative environmental impacts. While promising strategies to reduce methane emissions are being explored, including feed adjustments and additives (Hristov, 2024), genetic selection for low methane-producing sheep offers the potential of sustained long-term benefits (Worku, 2024). However, understanding of the genetic and biological mechanisms of methane production remains limited, with most research focused on rumen ecosystems (Asselstine et al., 2021) rather than live animals. This study aims to identify differentially expressed genes by utilizing blood transcriptome, exploring how methane emissions are related to physiological and metabolic pathways.

***Materials and Methods:*** Twenty-four male weaned lambs (Texel x Scottish Blackface, average weight 30 kg) were randomly assigned to four dietary groups, each receiving a total mixed ratio diet of 50:50 grass silage and concentrate, with varying amounts of microalgae oil (*Schizochytrium* sp.) supplementation levels: 0g, 5.4g, 10.8g, and 16.2g per kg dry matter. Dry matter intake (DMI) and body weight (BW) were recorded daily and weekly for nine weeks. Methane emissions were measured for 48 hours at the end of week 9 using individual respiration chambers. Blood samples collected at the end of experiment were analyzed via RNA sequencing, using an Illumina NovaSeq platform. Bioinformatic data analyses included sequence data quality checks, read alignment, normalization, differential expression analysis, weighted gene co-expression network analysis (WGCNA) and functional enrichment analysis. WGCNA module genes were further analyzed through protein-protein interaction network (PPI) construction to identify highly interconnected gene network associated with methane production.

***Results : :*** Microalgae oil supplementation had no significant effect on body weight (P= 0.25) or dry matter intake (DMI) (P= 0.07*),* indicating no impact of microalgae oil on growth or feed consumption. Methane emissions at the end of the study (week 9) varied between 19.50 and 47.86 g/d, negatively correlating with microalgae oil levels (R = -0.52, P = 0.01), suggesting microalgae oil effect in reducing methane emissions (Cristobal-Carballo et al., 2021). Differential expression analysis revealed seven significant genes linked to methane production, including upregulated *NME4, LOC121819234, MARCHF3*, and *PLXNB3*, and downregulated *LOC105603087, LOC132657460*, and *LOC101116551*. *NME4* and *PLXNB3* are involved in nucleotide metabolism and axonal guidance pathways. *NME4* upregulation, related to coenzyme-A (CoA) metabolism and lipid accumulation, was likely driven by microalgae oil supplementation, resulting in altered methane emissions. *PLXNB3* upregulation activates *PAK5 (P21 Activated Kinase 5),* a regulator of barrier function, and epithelial cell migration, also impacting the signalling pathways associated with gut inflammation. WGCNA revealed significant correlations between four gene modules (total of 3,671 genes) and methane emissions, with enriched pathways such as focal adhesion, efferocytosis and gap junctions. These pathways are crucial for cellular communication, inflammation resolution, and epithelial homeostasis, and have been reported to be influenced by diet by impacting nutrient absorption rates. Ten highly connected hub genes (*Histone H4(LOC101108868), Talin 1(TLN1)*, *Histone H3.1**(LOC101118484), Cofilin 1(CFL1),*  *Filamin A(FLNA), Calponin 2(CNN2), Actin gamma 1(ACTG1), Histone deacetylase 5(HDAC5), Histone H2A****X*** *(LOC101106791),* and *Actin beta (ACTB))* identified through PPI network analysis, were linked to histone and actin network families, which play roles in gene expression regulation, cell adhesion, and immune responses, and all positively correlated with methane production.

***Conclusion:*** This study has identified key genes associated with methane production in lambs, and involved in immune regulation, cellular differentiation, and cell adhesion. These findings offer insights into the molecular mechanisms of methane emissions and highlight candidate genes for marker-assisted selection of low methane-emitting sheep. Further research is needed to explore how these genes interact with microbiota-driven changes in the gut and to assess the long-term effects of microalgae oil supplementation on gene expression and methane emissions.

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