Borzouie, S., Jawad, H., Osonowo, O., Miar, Y., Manafiazar, G. (2026) A metabolomic perspective on the interaction between feed efficiency and parasite resistance in lambs, Proceedings 13th World Congress on Genetics Applied to Livestock Production 24377:
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A metabolomic perspective on the interaction between feed efficiency and parasite resistance in lambs

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Introduction

With the growing global demand for animal protein and increasing environmental pressures, enhancing the sustainability of livestock production is essential. The two main challenges to efficient small ruminant production are feed efficiency and parasitism. Gastrointestinal pathogens like *Haemonchus contortus* severely limit sheep production due to their blood-feeding nature and increasing resistance to parasite drug treatment, which reduces growth performance and, in severe cases, causes mortality (Besier et al., 2016; Emery et al., 2016). Residual Feed Intake (RFI) is used as an indicator of feed efficiency, allowing identification of more nutrient-efficient animals (low RFI) that consume less feed while achieving equivalent growth.

Because infections often remain subclinical and fecal egg counts fail to detect at the early disease stage, blood-based metabolic profiling offers a promising approach for earlier diagnosis and improved understanding of host-parasite interactions (Jawad et al., 2025), yet it could be used for identifying the efficient animals (Osonowo et al., 2025). The metabolomics approach analysis will be helping to explain how animals respond to stresses such as parasitic infection if they are feed efficient.

Our objective was to test the hypothesis that lambs with a high feed efficiency profile (low-RFI) have unique baseline and infection-responsive metabolic signatures compared to low feed efficient counterparts (high-RFI). By comparing high- and low-RFI individuals within the infected group, this work aims to identify potential candidate biomarkers and reveal key metabolic pathways associated with feed-efficient lamb responses to parasite resistance challenges. This provides a metabolomic perspective on the interaction between feed efficiency and parasite resistance. Applying this approach to feed efficiency during parasitic challenge provides a new way to identify the biochemical markers of feed efficiency and resistance, supporting more sustainable sheep production.

Materials and Methods

Experimental design. This study is part of a larger study (Jawad et al., 2025; Osonowo et al., 2025), where sixty parasite-free Rideau Arcott ewe lambs (6-7 months old; 44.6 ± 6.08 kg BW) were sourced from a commercial ranch to ensure genetic and management consistency and tested/phenotyped for RFI during 61 days trail (experiment I). Lambs were then subject to a parasite resistance challenge trial for a 57-day period by randomly assigning them into two groups: parasite-free control (CT, n = 30) receiving a sham solution, and *H. contortus*-inoculated (n = 30) receiving 5,000 L3 orally on day 0 (Experiment II). Each group was split further into treated and non-treated subgroups (15 lambs each); treated lambs received ivermectin and fenbendazole on day 28, and all lambs received toltrazuril on day 35 to target coccidia. This framed the experiment into two periods of infection (Day 1-28) and recovery (Day 28-57). Fecal egg counts were monitored (Jawad et al., 2025), and blood samples were collected at seven timepoints (days -1, 7, 21, 28, 35, 42, 57) of the trial. Blood serum was prepared, frozen, and stored at −80 °C until

analysis. Within the infected animals, on day 57, the RFI profiles were evaluated, and only parasite-resistant lambs were selected. High-RFI-resistant and low-RFI-resistant lambs were identified (n = 4 per group), resulting in two experimental groups for targeted serum metabolomic analysis. Targeted serum metabolomics was performed on these two groups (low-RFI-resistant parasite-challenged lambs and high-RFI-resistant parasite-challenged lambs), with analyses in three intervals: day 1 (baseline), days 1-28 (infection response), and days 35-57 (post-deworming recovery). This distinguished baseline, infection-induced, and recovery-associated metabolic signatures, capturing host-parasite interactions related to feed efficiency and resistance.

Metabolomics & Statistical Analysis. Serum metabolites were profiled using a targeted DI/LC–MS/MS assay quantifying up to 100 endogenous metabolites across major classes at the TMIC lab, University of Alberta. MetaboAnalyst 6.0 was used for both univariate and multivariate analyses, including Student's t-test, PLS-DA, and Random Forest (Pang et al., 2024). PLS-DA significance was assessed via permutation testing (n = 1000, $p < 0.05$), and the top 15 metabolites (Variable Importance in Projection: $VIP > 1$) were identified as main contributors to group separation. In Random Forest, important variables ranked in the top 10-20% of Mean Decrease Accuracy (MDA) scores. Significant metabolites ($p < 0.05$) were further evaluated as potential biomarkers using ROC curves, AUC (≥ 0.70) with 95% confidence intervals, and permutation testing (n = 1000, $p < 0.05$). Pathway and enrichment analyses were used to assess the metabolic impact of significant metabolites. Statistical significance was set at $p < 0.05$ ($Q < 0.05$), using false discovery rate (FDR) correction for multiple testing, where $0.05 \leq p < 0.10$ indicated a tendency, and $p > 0.10$ was considered non-significant, with standard errors considered for group means.

Results & Discussion

Targeted serum metabolomics quantified up to 108 metabolites spanning major biochemical classes, including amino acids, biogenic amines, organic acids, acylcarnitines, glycerophospholipids, and sphingolipids.

At the pre-infection baseline (day -1), eight candidate metabolites differentiated high- and low-RFI lambs, involving in five metabolic pathways, including arginine biosynthesis, the tricarboxylic acid (TCA) cycle, alanine aspartate glutamate metabolism, butanoate metabolism, and pyruvate metabolism. These metabolites and associated pathway were considered feed efficiency–related discriminating metabolites, given that lambs were not parasite- challenged at day -1. Different levels of metabolites, including α-ketoglutaric acid and fumaric acid, (Jiang et al., 2017) were observed in low-RFI lambs, indicating altered mitochondrial energy metabolism (Martínez-Reyes et al., 2020), while variations in indole acetic acid suggest changes in gut microbial activity (Dodd et al., 2017). These results show that feed efficient lambs have a unique metabolism even before parasite exposure, which may help them manage energy and nitrogen and better cope with future stress.

During the infection phase (days 1-28), 16 metabolites consistently distinguished RFI groups, showing that RFI-associated metabolic differences were still detectable during parasitic stress. Three common metabolites (LysoPC a C26:1, LysoPC a C20:4, and SM C16:1) were detected at both day -1 and days 1-28, suggesting that their metabolic regulation remains stable under stress. In contrast, 13 additional metabolites were detected only at the infection phase, indicating that feed efficiency may activate new or additional metabolic pathways to respond to the parasitic challenge. The response in low-RFI lambs was marked by differential concentrations of metabolites linked to integrated stress defense. Elevated taurine and polyamines (spermidine, spermine) suggested

enhanced antioxidant and cellular protection mechanisms, while shifts in butyrate-related metabolites pointed to better maintenance of gut health because butyrate supports intestinal barrier function and microbial balance. (Liu et al., 2007). These changes suggest a controlled immune-metabolic response to *H. contortus* infection. Altered acylcarnitines and lysophosphatidylcholines show that lambs adjust fatty acid use and membrane lipids, demonstrating metabolic flexibility and supporting natural resistance and efficient feed use.

Following deworming, the recovery phase revealed 13 potential biomarkers, highlighting that lambs recover metabolically in different ways. Three metabolites, LysoPC a C26:1, LysoPC a C20:4, and SM C16:1, were consistently present across all phases (baseline, infection response, and recovery), suggesting their levels are tightly regulated and not disrupted by parasitic stress. In contrast, a group of ten other metabolites, including specific amino acids, acylcarnitines, polyamines, and ketone bodies, emerged during the infection response phase and persisted into the recovery phase. This pattern defines a sustained infection-associated metabolic signature that differs from the normal baseline. The ongoing differences in β-hydroxybutyric acid indicates that energy use and metabolism have different levels during recovery after infection. Persistent variations in TMAO, lysophosphatidylcholines, and total dimethylarginine indicate that nitrogen metabolism and host-microbe interactions continue to adjust even after the parasites are cleared. This suggest that low-RFI lambs recover in different metabolic path, restoring balance while keeping metabolism stable. Because only four lambs were included per group, the following results highlight biologically relevant patterns and candidate biomarkers but estimates of effect size and statistical precision should be interpreted cautiously.

Conclusions

Overall, these results provide insight into the metabolic basis of feed efficiency and parasite resistance, showing that feed efficiency is more than a production trait; it reflects a coordinated, whole-body metabolic profile that interacts with infection. This interaction occurs across three stages: (1) before infection, efficient lambs show priming of energy and nitrogen metabolism; (2) during infection, they mount a controlled stress response that prioritizes redox balance, cellular protection, and metabolic flexibility; and (3) after infection, metabolic pathways diverge during recovery. This dynamic metabolic interplay gives efficient lambs an advantage in managing and recovering from parasitic challenge. Importantly, the metabolites identified represent potential biomarkers of feed efficiency and parasite resistance, offering opportunities to inform nutritional and breeding strategies aimed at improving productivity and supporting more sustainable sheep production.

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