Research Article

Plasma Metabolomics Identifies Nutritional Biomarkers in Tropical Saanen Goats

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Abstract | Nutritional adequacy is a major determinant of productivity in dairy goats, however, conventional intake and digestibility measurements provide limited insight into the biochemical and physiological adaptations underlying nutrient utilization because they primarily quantify feed consumption and apparent digestibility without capturing internal metabolic adjustments or stress-related responses. Metabolomics offers a complementary approach to identify biomarkers of nutritional status by providing pathway-level information on metabolic processes, which is particularly relevant for Saanen goats raised under tropical conditions characterized by heat stress and variable forage quality. This study aimed to integrate feed intake and digestibility parameters with plasma metabolomic profiling to identify biomarker candidates of nutritional status in Saanen goats raised in Indonesia. Twelve clinically healthy, non-lactating adult Saanen goats were assigned to low, medium, and high nutrient groups. Dry matter intake (DMI), organic matter intake (OMI), dry matter digestibility (DMD), and organic matter digestibility (OMD) were measured, and plasma metabolites were profiled using gas chromatography–mass spectrometry (GC–MS). Data were analyzed using multivariate statistics, analysis of variance, pathway enrichment, and receiver operating characteristic analyses. OMI and OMD were more sensitive than DMI and DMD in differentiating nutritional status among groups. Metabolomic analyses revealed clear group-specific clustering, with discriminant metabolites linked to carbohydrate, amino acid, and energy metabolism. Six metabolites such as citric acid, nicotinic acid, D-fructose, threonine, palmitic acid, and glycerol showed strong discriminatory capacity and significant correlations with OMI and OMD, supporting their physiological relevance to nutrient utilization. In conclusion, integrating metabolomic profiling with conventional intake and digestibility indices provides a more comprehensive assessment of nutrient utilization in Saanen goats under tropical conditions and supports the identification of biomarkers relevant for nutritional monitoring and precision feeding strategies to enhance sustainable dairy goat production.3

Keywords | Saanen goats, Metabolomics, Feed intake and digestibility, Biomarker, Tropical nutrition

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INTRODUCTION

Goats are a vital component of smallholder farming systems in tropical regions, contributing substantially to food security, household income, and rural resilience (Ruvuga and Maleko, 2023). The Saanen goat, a dairy breed of temperate origin, has been widely introduced into Indonesia for its high milk production potential (Sumarmono, 2022). However, the breed's adaptation to tropical environments is constrained by continuous heat load, high humidity, and the generally low nutritive value of locally available forages, all of which compromise feed efficiency and productivity (De Vasconcelos *et al*., 2021; Lima *et al*., 2022). These environmental stressors not only reduce nutrient intake and digestibility but also alter energy and amino acid metabolism, leading to oxidative and thermal stress that disrupt metabolic homeostasis. In the present study, these climatic stressors were treated as inherent background conditions of the production environment rather than experimentally controlled variables, reflecting typical smallholder systems in Indonesia.

Conventional nutritional assessments in ruminants rely on dry matter intake (DMI), organic matter intake (OMI), and digestibility indices such as dry matter digestibility (DMD) and organic matter digestibility (OMD) (Colombo *et al*., 2021; NRC, 2007). While these parameters are essential for evaluating feed utilization, they primarily describe intake and apparent digestion and do not capture the internal metabolic responses through which animals adapt to nutritional constraints under tropical conditions. When thermal stress is relatively constant across animals, variation in intake and digestibility may be accompanied by metabolic adjustments that are not detectable using conventional indices alone. As a result, nutritional responses may be confounded with physiological stress responses, leading to an incomplete understanding of nutrient utilization efficiency (Tran *et al*., 2020). Metabolomics offers such an approach by providing a comprehensive profile of metabolites that reflect physiological responses to dietary intake and environmental stressors (Brennan and Roos, 2023; Singh *et al*., 2024). In dairy cattle, metabolomics has been applied to identify plasma biomarkers of feed efficiency, energy balance, and metabolic disorders (Pires *et al*., 2022; He *et al*., 2025). In goats, research has mainly focused on feed supplementation strategies, milk composition, or stress responses (Batchu *et al*., 2021; Abdelkrim *et al*., 2023). However, relatively little attention has been given to the integration of feed intake and digestibility parameters with plasma metabolomic profiles, particularly in Saanen goats raised under tropical conditions. The integration of feed intake and digestibility parameters with plasma metabolomic profiles particularly in Saanen goats raised under tropical conditions remains limited.

This integrative approach is particularly needed in Indonesia, where unique climatic and forage challenges create a production environment distinct from temperate systems. Identifying metabolic signatures that correspond to nutrient intake and digestibility under such conditions is critical to improving nutritional monitoring and precision feeding strategies. In this study, the hypothesis that metabolomic signatures extend beyond those detectable by conventional nutritional indices does not imply superiority in predicting production or performance outcomes. Rather, it posits that metabolomics can reveal diet-associated metabolic patterns that are not directly inferred from intake and digestibility values alone. Evidence against this hypothesis would include the absence of distinct metabolomic patterns across nutritional groups or a lack of association between identified metabolites and intake–digestibility parameters. Accordingly, this hypothesis-driven yet exploratory study evaluates whether variation in DMI, OMI, DMD, and OMD under a common thermal background is associated with distinct plasma metabolomic signatures, without claiming predictive validity for functional outcomes such as growth or stress resilience, which were beyond the scope of the present design.

MATERIALS AND METHODS

Animals and experimental design

Twelve clinically healthy Saanen goats were used in this study. The animals were 24–48 months of age, with an initial body weight of 35–55 kg and a body condition score (BCS) of 2–4 on a 1–5 scale. All goats were non-lactating (dry) throughout the experimental period; therefore, milk yield was not applicable. The goats were obtained from the Teaching Farm, Faculty of Animal Science, Universitas Hasanuddin, Makassar, Indonesia.

Parity status was balanced across treatments, with two multiparous and two nulliparous goats per dietary group goats per dietary group, to minimize potential confounding effects of reproductive history on plasma metabolomic profiles. Goats were randomly assigned to three dietary treatment groups (n= 4 per group): low, medium, and high nutrient diets.

All diets were formulated based on NRC (2007) maintenance requirements, targeting approximately 80%, 100%, and 120% of recommended nutrient supply for the low, medium, and high groups, respectively. The 120% maintenance level was designed to represent a mild nutritional surplus rather than ad libitum feeding, consistent with practical management of non-lactating goats to support body condition and metabolic reserves without inducing excessive weight gain. The dietary treatments were prepared using the same basal feed ingredients, consisting primarily of Napier grass and a

concentrate mixture (rice bran, corn, soybean meal, and mineral premix). Nutrient density (crude protein and energy) was adjusted quantitatively by modifying the inclusion levels/ratio of forage and concentrate while maintaining the same ingredient sources across treatments. This standardized formulation minimized confounding effects related to feed type or ingredient-specific metabolites, allowing observed metabolomic differences to be interpreted primarily in relation to graded nutrient supply, while accounting for individual physiological and genetic variation under tropical management conditions. Although the total sample size was limited (n= 12), the study was designed as a hypothesis-driven but exploratory investigation rather than a definitive biomarker validation study. Multivariate analyses (PCA and PLS-DA) were used primarily for pattern recognition were applied for pattern recognition and hypothesis generation, with interpretation restricted to consistent group-level trends and biologically plausible pathway enrichment, rather than individual-level prediction. Group separation in multivariate space was therefore interpreted as indicative of potential metabolic differentiation associated with nutrient supply, not as conclusive evidence of robust biomarker performance. Accordingly, the metabolites identified are presented as candidate biomarkers that require confirmation and validation in larger, independent cohorts. All animals were managed under standard husbandry practices. Ethical approval was obtained from the Animal Ethics Committee of Universitas Hasanuddin.

Feed intake and digestibility parameters

Feed intake and digestibility were evaluated by measuring dry matter intake (DMI), organic matter intake (OMI), dry matter digestibility (DMD), and organic matter digestibility (OMD) (Woodmartin *et al*., 2024). Feed offered to each goat was weighed daily, and refusals were collected and measured after 24 hours. The difference between the feed provided and the residuals represented the daily DMI, while OMI was calculated by considering the organic matter content of both the feed and refusals. To determine digestibility, feed samples were ground to pass a 1-mm sieve and analyzed using an *in vitro* digestubility method. Rumen fluid was collected from donor goats, filtered through cheesecloth, and used as an inoculum. All donor goats were maintained on the same medium-nutrient diet for two weeks before sampling to standardize rumen microbial activity and minimize inoculum-related variability across treatments.. Approximately 1 g of feed sample was incubated with the buffered rumen fluid in sealed fermentation tubes at 39 °C for 48 h under anaerobic conditions. Following incubation, residues were subjected to enzymatic digestion using a pepsin-HCl solution (pH 2.0) for an additional 48 h to simulate abomasal digestion. After filtration and drying, the residues were weighed to calculate DMD and OMD, with the latter adjusted for ash content to obtain organic matter values. These procedures provided reliable estimates of feed intake and nutrient digestibility under different dietary treatments.

Digestibility values derived from this procedure represent in vitro estimates and were not directly calibrated against *in vivo* digestibility measurements for the experimental diets used in this study. The in vitro technique was therefore applied as a standardized and widely accepted comparative method to assess relative differences in digestibility among dietary treatments rather than to generate absolute estimates of *in vivo* nutrient utilization. Accordingly, DMD and OMD values were interpreted in relative terms within the experimental context, allowing consistent comparison of dietary treatments under controlled laboratory conditions while minimizing animal-to-animal variability. The absence of diet-specific *in vivo* calibration is acknowledged as a methodological limitation, and its potential implications for interpretation are explicitly discussed in the Discussion section.

Rumen fluid inoculum was obtained from donor goats maintained on the medium-nutrient diet and pooled prior to use to standardize microbial activity across all in vitro incubations. This approach was intentionally adopted to minimize variability associated with donor-specific or diet-specific rumen microbial adaptation, thereby allowing digestibility differences to be interpreted relative to feed characteristics and nutrient supply rather than inoculum effects. Consequently, *in vitro* digestibility outcomes were evaluated as comparative indicators among dietary treatments, acknowledging that the use of a single standardized inoculum may not fully reflect diet-adapted rumen microbial ecology under *in vivo* conditions.

Blood sampling and metabolomic analysis

Blood samples were collected from each goat via jugular venipuncture into EDTA-coated tubes to prevent clotting (Huang *et al*., 2023). Samples were immediately placed on ice and transported to the laboratory for processing. Plasma was separated by centrifugation at 13,000 rpm for 20 minutes at 4°C, and the supernatant was carefully transferred into 1.5 mL microtubes. Each goat represented one biological replicate, resulting in a total of 12 biological replicates (n= 4 per dietary treatment). Aliquots of 50 µL plasma were mixed with 150 µL methoxylamine hydrochloride in methanol (1 mg/mL, used as an derivatization reagent; Sigma-Aldrich, St. Louis, USA) and 350 µL extraction solution (water: methanol, 1:4 v/v). The mixture was vortexed for 1 minute to ensure homogenization and centrifuged again at 13,000 rpm for 20 minutes at 4 °C. The supernatant was filtered through a 25 mm syringe filter, transferred into a clean microtube, and evaporated to dryness using a vacuum evaporator at

36°C for approximately 2 hours (Zhang *et al*., 2025a).

For derivatization, 100 µL of N-Trimethylsilyl-N-methyl trifluoroacetamide with 1% trimethylchlorosilane (MSTFA + 1% TMCS; Sigma-Aldrich, USA) was added to the dried extract. Samples were heated in a water bath at 70°C for 1 hour, then centrifuged at 13,000 rpm for 10 minutes at 4°C. The clear supernatant containing derivatized metabolites was transferred into 2 mL amber glass vials for analysis (Xu *et al*., 2023). To ensure analytical reproducibility, pooled quality control (QC) samples were prepared by combining equal aliquots of all plasma extracts and analyzed at regular intervals throughout the batch.

Metabolomic profiling was performed using a Shimadzu GCMS-QP2010 system equipped with an SH-RI-5MS capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness; Agilent Technologies, Santa Clara, CA). One microliter of each derivatized sample was injected in splitless mode with helium (99.9% purity) as the carrier gas at a constant flow rate of 3 mL/min. The GC oven was programmed as follows: initial temperature 80°C held for 2 min, ramped at 10°C/min to 325°C, and held for 6 min. The injector temperature was set at 270°C, interface temperature at 260°C, and ion source at 200°C. Electron ionization was performed at 70 eV with a mass scan range of m/z 30–600. Metabolite identification was achieved by comparing acquired spectra with the NIST 20 mass spectral library and confirmed using retention indices obtained from the NIST Chemistry WebBook. Only metabolites with consistent detection across QC samples were retained for further statistical analysis (Samir *et al*., 2023).

Statistical analysis

Data on feed intake and digestibility parameters (DMI, OMI, DMD, OMD) were analyzed using one-way ANOVA with Tukey's HSD post-hoc test to determine significant differences among dietary treatments. Statistical significance was declared at $P < 0.05$, and results are presented as means ± standard deviation. For metabolomic profiling, data were normalized, log-transformed, and subjected to principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) was subsequently used as an exploratory tool for pattern recognition rather than confirmatory inference. Metabolites with Variable Importance in Projection (VIP) > 1.0 were considered as potential biomarkers. To reduce the risk of Type I error due to multiple testing, metabolite-wise p-values were adjusted using false discovery rate (FDR) correction (Benjamini–Hochberg procedure), and we focused on biologically plausible and pathway-consistent metabolites. Pathway enrichment and topology analysis were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) to determine the biological significance of identified metabolites. Pathway impact values were generated using the MetaboAnalyst 6.0 pathway topology module based on relative betweenness centrality. To further evaluate the discriminative potential of selected candidate metabolites, receiver operating characteristic (ROC) curve analysis was conducted in an exploratory manner, and the area under the curve (AUC) was reported as a measure of classification performance. Given the limited sample size, ROC results were interpreted cautiously and are presented as indicative of potential discriminatory ability rather than as validated diagnostic performance.

All statistical analyses were performed using SPSS v20 (IBM Corp., USA) for intake and digestibility data, and MetaboAnalyst 6.0 (www.metaboanalyst.ca) for metabolomic and pathway analyses.

RESULTS

Feed intake and digestibility

As summarized in Table 1, dry matter intake (DMI) and dry matter digestibility (DMD) did not differ significantly among groups ($p > 0.05$). Organic matter intake (OMI) was significantly higher in the High compared to the Low group ($p < 0.05$), whereas the Medium group did not differ from either. Organic matter digestibility (OMD) differed significantly among all groups ($p < 0.05$).

Table 1: Classification of nutritional status of Saanen goat based on feed intake and digestibility.

Group	DMI (g.day)	OMI (g/day)	DMD (%)	OMD (%)
Low	1165.0±15.0^{a}	982.7±13.51^{a}	61.8±4.7^{a}	61.0±0.8^{a}
Medium	1172.5±11.2^{a}	1030.1±24.3^{ab}	66.9±0.6^{a}	69.5±0.4^{b}
High	1181.7±12.6^{a}	1123.8±24.4^{b}	71.2±4.7^{a}	77.4±1.0^{c}

Note: Different superscripts within the same column indicate significant differences among groups ($p < 0.05$).

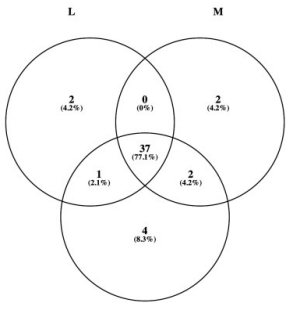

Figure 1: Venn diagram showing the distribution of identified metabolites in nutritional status groups across low, medium, and high-nutrient dietary groups of Saanen goats after quality control of Saanen goats after quality control (QC).

Metabolomic profile

As presented in Figure 1, a total of 37 metabolites (77.1%) were shared among all groups after quality control (QC)

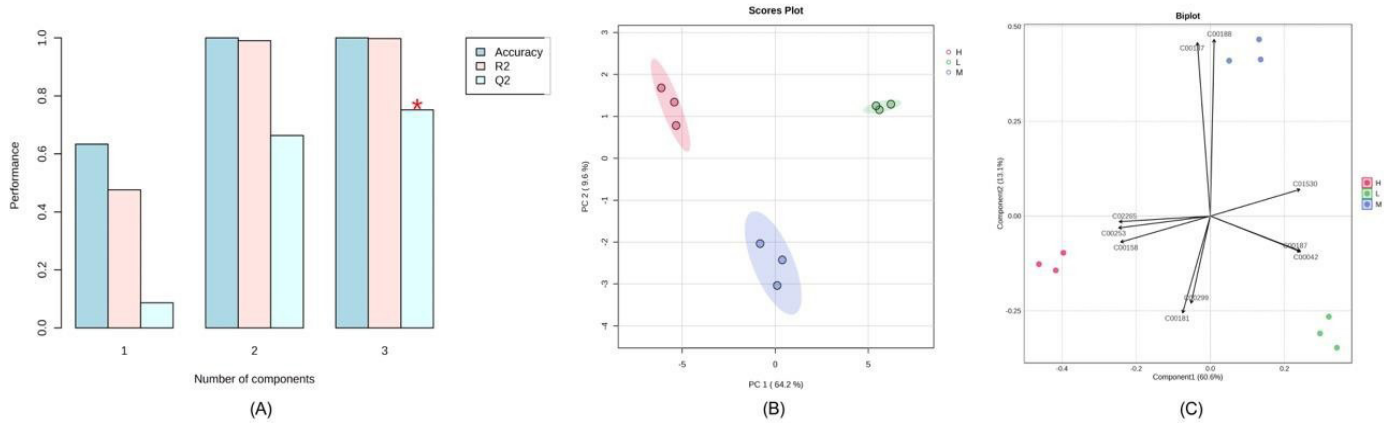

Figure 2: Multivariate analysis of metabolite profiles in nutritional status groups of Saanen goats. (A) PCA Score Plot. (B) PLSDA Biplot. (C) Cross Validation.

based on KEGG ID. In contrast, a limited number of unique metabolites were detected in each group, with four metabolites specific to the High group, two to the medium group, and two to the Low group. A small number of metabolites were shared exclusively between groups, indicating both common and group-specific metabolic features.

Differential metabolite analysis

As shown in Figure 2A, Principal Component Analysis (PCA) revealed a clear separation among the Low, Medium, and High groups, indicating distinct clustering patterns of metabolite profiles. The first two principal components (PC1 and PC2) explained 46.3% and 21.7% of the total variance, respectively, confirming that most metabolic variation among groups was captured by these components. To further enhance group discrimination, a supervised Partial Least Squares Discriminant Analysis (PLS-DA) was performed. The PLS-DA biplot (Figure 2B) highlighted key metabolites contributing to the separation across groups. Cross validation (Figure 2C) confirmed the robustness of the analysis, with high accuracy, R^2, and Q^2 values supporting the reliability of the PLS-DA model ($p < 0.05$). The PLS-DA model showed good performance, with R^2= 0.89 and Q^2= 0.73, indicating strong model fit and predictive ability.

As shown in Figure 3, hierarchical clustering revealed distinct metabolite expression patterns across groups. The High, Medium, and Low groups formed separate clusters, indicating clear metabolic differentiation. Several metabolites were up-regulated in the High group but down- regulated in the Medium or Low groups, and vice versa, supporting the group separation observed in PCA and PLS-DA analyses.

As shown in Figure 4, several metabolite features displayed significant differences among groups, with one metabolite emerging as highly significant ($p < 0.001$). The list of

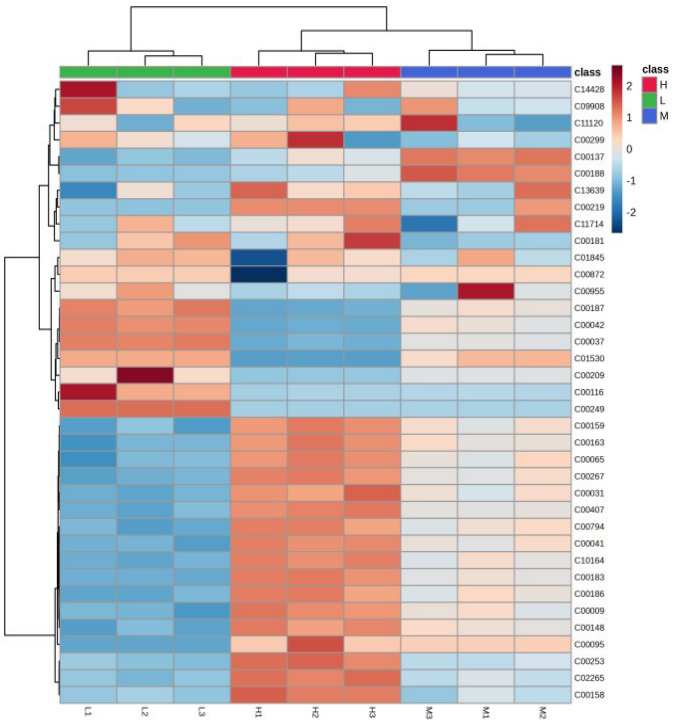

Figure 3: Heatmap with hierarchical clustering of metabolites in nutritional status groups of Saanen goats.

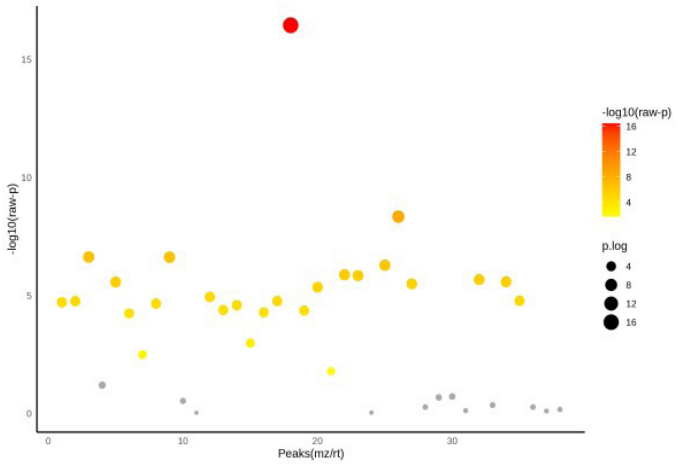

Figure 4: Anova plot of detected metabolites in nutritional status groups of Saanen goats.

Table 2: Differentially expressed metabolites in nutritional status groups of Saanen goats.

KEGG ID	p-value	FDR (False Discovery Rate)	Fisher's LSD
C00249	3.53E-13	1.3E-11	L vs H; M vs H; L vs M
C00037	4.54E-05	8.6E-04	L vs H; M vs H; L vs M
C00183	2.33E-03	2.3E-02	H vs L; H vs M; M vs L
C00042	2.37E-03	2.3E-02	L vs H; M vs H; L vs M
C00407	5.22E-03	4.0E-02	H vs L; H vs M; M vs L
C00187	1.32E-02	7.8E-02	L vs H; M vs H; L vs M
C10164	1.43E-02	7.8E-02	H vs L; H vs M; M vs L
C00041	2.10E-02	1.0E-01	H vs L; H vs M; M vs L
C02265	2.60E-02	1.0E-01	H vs L; H vs M; M vs L
C00267	2.67E-02	1.0E-01	H vs L; H vs M; M vs L
C00253	3.19E-02	1.1E-01	H vs L; H vs M; M vs L
C01530	4.45E-02	1.4E-02	L vs H; M vs H
C00148	1.15E-01	3.4E-01	H vs L; H vs M; M vs L
C00009	1.67E-01	4.1E-01	H vs L; H vs M; M vs L
C00794	1.72E-01	4.1E-01	H vs L; H vs M; M vs L
C00186	1.73E-01	4.1E-01	H vs L; H vs M; M vs L
C00163	1.94E-01	4.3E-01	H vs L; H vs M; M vs L
C00188	2.19E-01	4.6E-01	H vs L; M vs H; M vs L
C00159	2.57E-01	5.1E-01	H vs L; H vs M; M vs L
C00158	4.11E-01	7.8E-01	H vs L; H vs M
C00137	4.34E-01	7.8E-01	H vs L; M vs H; M vs L
C00031	5.15E-01	8.9E-01	H vs L; H vs M; M vs L
C00065	5.73E-01	9.5E-01	H vs L; H vs M; M vs L
C00095	1.03E-03	1.6E-03	H vs L; M vs L
C00116	3.15E-03	4.8E-03	L vs H; L vs M
C00219	1.61E-02	2.4E-02	H vs L; H vs M

Note: L= Low nutrient diet; M= Medium nutrient diet; H= High nutrient diet.

discriminant metabolites is presented in Table 2, where C00249 exhibited the strongest significance, followed by C00037 and C00095. In addition, several other KEGG IDs demonstrated significant variation across the Low, Medium, and High groups ($p < 0.05$), supporting their role as key contributors to metabolic differentiation. Although several metabolites showed nominal p-values < 0.05, many did not remain statistically significant after FDR correction (FDR > 0.1) and are therefore interpreted as exploratory findings.

As shown in Figure 5, the PLS-DA model identified the top 15 discriminant metabolites ranked by their VIP scores. Among these, 11 metabolites displayed VIP values greater than 1, indicating their strong contribution to group separation. C00188 and C00137 were the highest ranked features, followed by C00181, C00249, and C00116, while the remaining metabolites contributed moderately to the differentiation of nutritional status groups in Saanen goats.

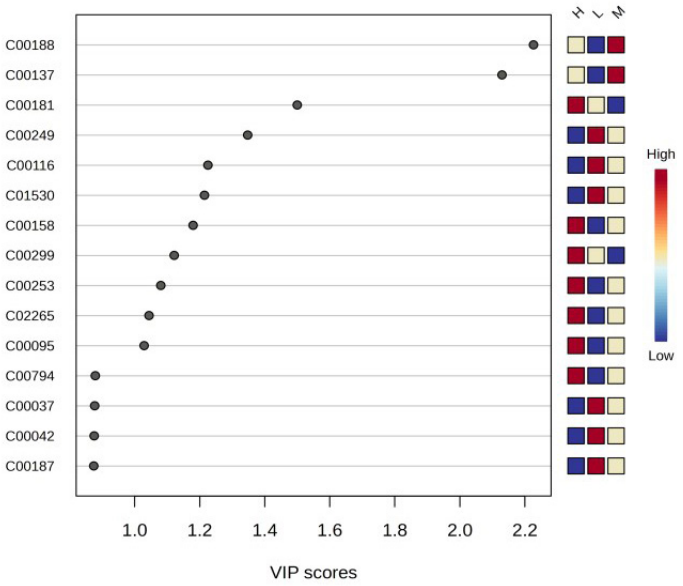

Figure 5: Variable Importance in Projection (VIP) scores of discriminant metabolites in nutritional status groups of Saanen goats.

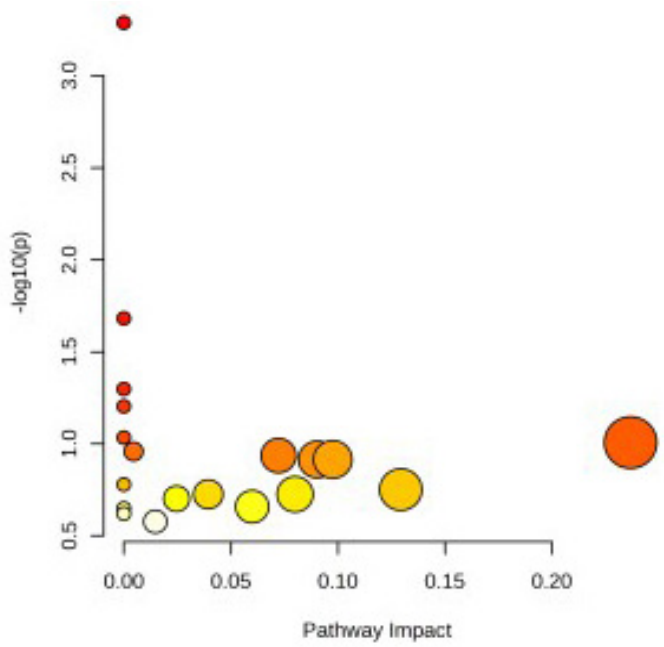

Figure 6: Pathway enrichment analysis of discriminant metabolites in nutritional status groups of Saanen goats.

Pathway enrichment analysis

As shown in Figure 6 and Table 3, galactose metabolism was the only pathway that remained potentially enriched after FDR correction (FDR= 0.041). All other pathways showed high FDR values (≥ 0.833), indicating no statistically significant enrichment. Nevertheless, several of these pathways exhibited non-zero topology impact scores, reflecting that one mapped metabolite occupied a relatively central position within the pathway network.

Table 3: Pathway enrichment analysis of discriminant metabolites in nutritional status groups of Saanen goats.

Pathway	Total	Hits	FDR (False Discovery Rate)	Impact	KEGG ID
Galactose metabolism	27	3	0.041	0	C00137; 00116; C00095
Biosynthesis of unsaturated fatty acids	36	2	0.833	0	C00249; C01530
Valine, leucine and isoleucine biosynthesis	8	1	0.973	0	C00188
Ascorbate and aldarate metabolism	10	1	0.973	0	C00137
Nicotinate and nicotinamide metabolism	15	1	0.973	0.237	C00253
Glycerolipid metabolism	16	1	0.973	0.005	C00116
Starch and sucrose metabolism	18	1	0.973	0.072	C00095
Pentose and glucuronate interconversions	19	1	0.973	0.090	C00181
Citrate cycle (TCA cycle)	20	1	0.973	0.098	C00158
Fructose and mannose metabolism	20	1	0.973	0	C00095
Alanine aspartate and glutamate metabolism	28	1	0.998	0.039	C00158
Inositol phosphate metabolism	30	1	0.998	0.039	C00095
Glycosyphosphatidylinositol (GPI)-anchor biosynthesis	32	1	0.998	0.080	C00249
Glyoxylate and dicarboxylate metabolism	32	1	0.998	0.025	C00158
Glycine, serine and threonine metabolism	34	1	0.998	0.060	C00188
Pyrimidine metabolism	38	1	0.998	0	C00299
Fatty acid elongation	39	1	0.998	0	C00249
Fatty acid degradation	39	1	0.998	0	C00249
Amino sugar and nucleotide sugar metabolism	42	1	1.000	0.015	C00095
Fatty acid biosynthesis	47	1	1.000	0.01	C00249

Table 4: Biomarker identification of discriminant metabolites in nutritional status groups of Saanen goats.

KEGG ID	Name	Group (Dominant)	VIP	Pathway	AUC
C00188	L-threonine	M	2.2266	Glycine, serine and threonine metabolism	1.0
C00181	D-Xylose	H	1.4999	Pentose and glucuronate interconversions	0.8
C00249	Palmitic acid	L	1.3475	Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1.0
C00116	Glycerol	L	1.2253	Glycerolipid metabolism	1.0
C00158	Citric acid	H	1.1797	Citrate cycle (TCA cycle); Alanine, aspartate and glutamate metabolism; Glyoxylate and dicarboxylate metabolism	1.0
C00253	Nicotinic Acid	H	1.0804	Nicotinate and nicotinamide metabolism	1.0
C00095	D-Fructose	H	1.029	Starch and sucrose metabolism; Inositol phosphate metabolism	1.0

These impact values should be interpreted cautiously and are presented as exploratory observations rather than evidence of significant pathway perturbation, including nicotinate and nicotinamide metabolism (Impact= 0.237; C00253), starch and sucrose metabolism (Impact= 0.072; C00095), pentose and glucuronate interconversions (Impact= 0.090; C00181), citrate cycle (TCA cycle) (Impact= 0.098; C00158), glycine, serine and threonine metabolism (Impact= 0.060; C00188), and inositol phosphate metabolism (Impact= 0.039; C00095).

ROC analysis and biomarker identification

As shown in Table 4 and Figure 7, biomarker candidates were identified using the combined criteria of VIP > 1, measurable pathway impact, and ROC analysis with AUC = 1.0. While several metabolites achieved an AUC of 1.0, this perfect discrimination may partly reflect the small sample size and possible model overfitting, and therefore these biomarkers should be validated in a larger population. A total of six biomarker candidates (C00188, C00249, C00116, C00158, C00253, and C00095) were confirmed to differentiate the nutritional status groups of Saanen goats. Among the identified metabolites, D-Xylose showed a slightly lower AUC value (0.8), indicating moderate discriminatory ability compared to other metabolites. This may reflect greater biological variability in carbohydrate-related metabolites under tropical feeding conditions. The High (H) group was characterized by C00158 (citric acid),

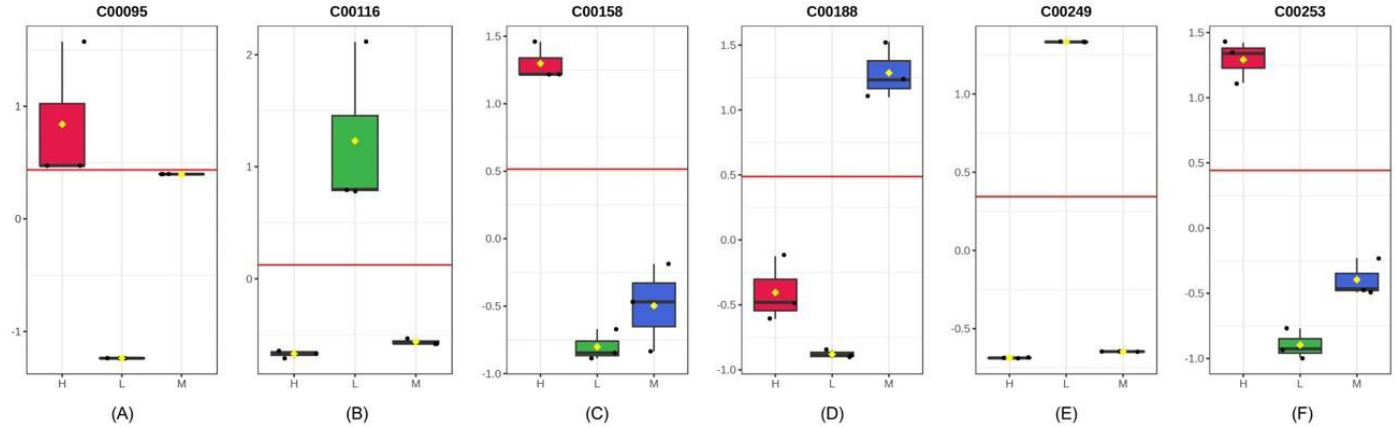

Figure 7: Boxplots of candidate biomarker metabolites identified in nutritional status groups of Saanen goats.

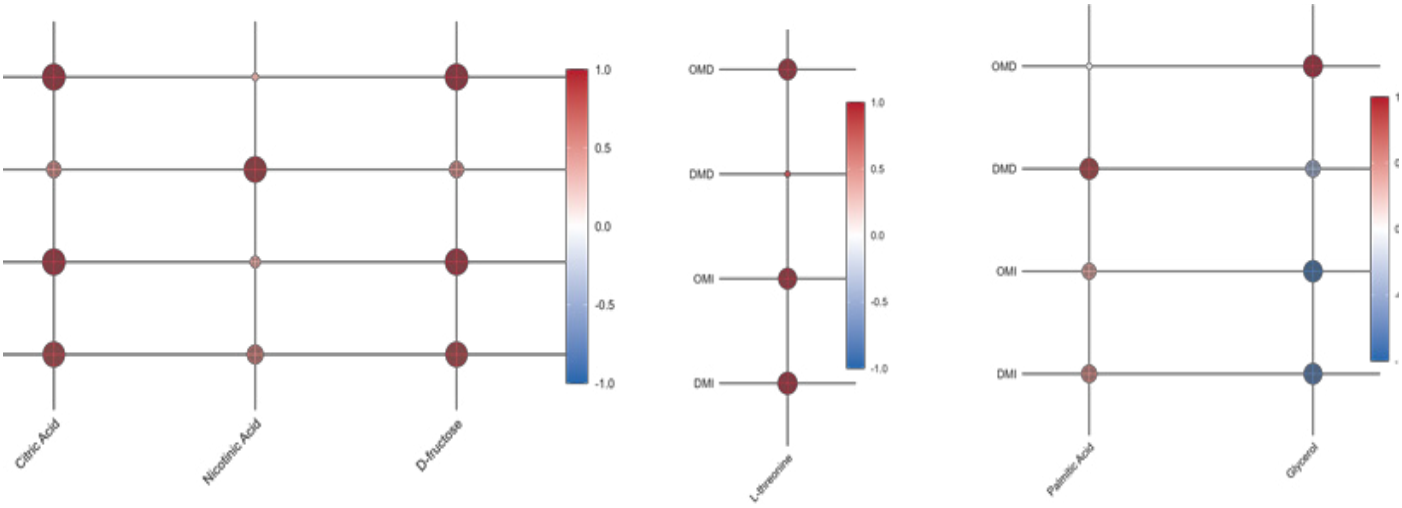

Figure 8: Pearson correlation heatmaps between feed intake and digestibility with biomarker candidates in nutritional status groups of Saanen goats.

C00253 (nicotinic acid), and C00095 (D-fructose) (Figure 7A, C, F), the Low (L) group by C00249 (palmitic acid) and C00116 (glycerol) (Figure 7B, E), and the Medium (M) group by C00188 (L-threonine) (Figure 7D). These biomarker candidates exhibited distinct group-specific expression patterns, underscoring their role in the metabolic differentiation among nutritional status groups.

Correlation between feed intake, digestibility and biomarker candidates

Figure 8A–C presents the correlation heatmaps between feed intake parameters (DMI, OMI) and digestibility indices (DMD, OMD) with the detected metabolite biomarkers in the High, Medium, and Low groups, respectively. In the High group (Figure 8A), citric acid and D- fructose showed very strong positive correlations with DMI, OMI, and OMD ($r > 0.90$), while nicotinic acid exhibited the strongest correlation with DMD ($r = 0.95$). In the Medium group (Figure 8B), L-threonine displayed consistently strong positive correlations with all feed intake and digestibility parameters, with coefficients ranging from $r = 0.87$ to 0.99. In the Low group (Figure 8C), palmitic acid was strongly correlated with DMD ($r = 0.91$), whereas glycerol showed negative correlations with DMI and OMI ($r = -0.87$ to -0.94) but a strong positive correlation with OMD ($r = 1.00$).

DISCUSSION

Nutrient utilization in dairy goats is strongly influenced by dietary quality and environmental conditions, with tropical climates posing particular challenges due to heat stress and fluctuating forage nutritive value. Saanen goats, being of temperate origin, are particularly sensitive to such stressors, which may compromise feed efficiency and metabolic resilience (Lima *et al*., 2022; Parsad *et al*., 2025). In the present study, the classification of goats into low, medium, and high nutritional status groups was based on intake and digestibility parameters (Table 1), where organic matter intake (OMI) and organic matter digestibility (OMD) provided clearer discrimination compared with dry matter intake (DMI) and dry matter digestibility (DMD). Similar findings were reported in dairy cattle, where organic matter-based measures were

better predictors of energy availability and performance under varying diet quality than total dry matter intake (Tedde *et al*., 2021). This suggests that OMI and OMD represent more sensitive nutritional indices under tropical conditions, where forage quality is inconsistent.

Metabolomic profiling further distinguished the nutritional groups, complementing classical nutritional indices. After quality control, the majority of detected metabolites were shared among groups, but a subset remained unique to specific groups, as shown in the Venn diagram (Figure 1). This reflects both conserved core metabolism and group-specific biochemical adjustments. Multivariate analyses, including PCA and PLS-DA (Figure 2), demonstrated clear clustering, indicating that differences in intake and digestibility were mirrored in plasma metabolite profiles. Comparable results have been observed in dairy cattle, where PCA and PLS-DA separated animals by feed efficiency or energy balance (Touitou *et al*., 2022; James *et al*., 2024). The heatmap with hierarchical clustering (Figure 3) reinforced this separation, consistent with earlier metabolomic studies in goats that reported diet-specific clustering of metabolite patterns (Batchu *et al*., 2021). These results support the utility of metabolomics as a pattern-recognition tool that complements, rather than replaces, conventional intake and digestibility measures.

The analysis of differentially expressed metabolites provided additional insight into the biochemical basis of this separation. ANOVA plots and post-hoc tests (Figure 4, Table 2) revealed that palmitic acid, glycerol, and glycine, along with simple sugars such as D- fructose, varied significantly among groups. Palmitic acid and glycerol were interpreted as indicators of lipid-related metabolic responses under lower nutrient availability; however, it is acknowledged that these metabolites are also influenced by dietary lipid supply and rumen biohydrogenation. Importantly, all experimental diets were formulated using the same basal ingredients and comparable fat content, thereby reducing but not eliminating the likelihood that observed plasma differences were driven solely by dietary composition. In the absence of direct measurements of body fat mobilization (e.g., non-esterified fatty acids or body weight change), the interpretation of these metabolites is therefore cautious and limited to their association with nutritional status rather than definitive evidence of endogenous lipid mobilization. Similar plasma fatty acid responses have been reported in cattle experiencing negative energy balance or dietary restriction (Mekuriaw, 2023; Młynek *et al*., 2021), while amino acids such as glycine have been linked to protein metabolism and rumen microbial activity in goats (Wu *et al*., 2023). The detection of carbohydrate metabolites such as D-fructose and glucose as discriminants agrees with studies in dairy sheep, where carbohydrate flux was directly associated with feed efficiency (Suárez-Vega *et al*., 2023).

The discriminant role of these metabolites was further supported by Variable Importance in Projection (VIP) scores from the PLS-DA model. As shown in Figure 5, eleven of the top fifteen metabolites had VIP values greater than 1, underscoring their importance in differentiating nutritional groups. Threonine, myo-inositol, and palmitic acid were among the most influential features, consistent with reports in dairy cattle where amino acid metabolism, carbohydrate regulation, and fatty acid mobilization explained much of the variance in feed efficiency (Daddam *et al*., 2025). These findings indicate that metabolomic features enhance discrimination among nutritional groups at the multivariate level, rather than serving as independent predictive indices of performance.

Pathway enrichment analysis contextualized these metabolite differences within broader biological processes. As presented in Figure 6 and Table 3, several pathways exhibited measurable impact values, including nicotinate and nicotinamide metabolism, starch and sucrose metabolism, pentose and glucuronate interconversions, the citrate cycle, and glycine, serine and threonine metabolism. Although not statistically significant after FDR correction, these pathways have measurable impact values and mirror those previously implicated in nutritional stress responses. For example, nicotinate metabolism is critical for NAD^+ biosynthesis and redox balance in ruminants (Wei *et al*., 2021), while the citrate cycle is a central hub of energy metabolism affected by dietary quality (Wang and Lou, 2024). Although several discriminant metabolites were strongly correlated with OMI and OMD, metabolomics adds value by providing insight into the underlying physiological processes associated with intake and digestibility, rather than simply duplicating conventional nutritional indicators. While OMI and OMD quantify nutrient intake and digestion, they do not capture post-absorptive metabolic allocation or stress-related prioritization, which are critical under tropical conditions. Metabolomic profiling revealed coordinated shifts in mitochondrial energy metabolism, redox balance, and substrate partitioning between carbohydrate and lipid utilization, indicating targeted metabolic adjustments that enhance resilience to combined heat and nutritional stress. Thus, metabolomics complements conventional measures by providing pathway-level insight into tropical metabolic adaptation rather than independent nutritional indices. (Lima *et al*., 2022; Parsad *et al*., 2025). The detection of glycine, serine, and threonine metabolism is also consistent with earlier studies in goats that linked amino acid turnover to dietary protein adequacy (Cao *et al*., 2021).

The integration of statistical and biological evidence led to the identification of six biomarker candidates with

strong discriminatory capacity. As summarized in Table 4 and illustrated in Figure 7, citric acid, nicotinic acid, and D-fructose characterized the high group, reflecting enhanced energy metabolism and carbohydrate flux; palmitic acid and glycerol were markers of the low group, indicating reliance on lipid mobilization under nutrient limitation; and threonine characterized the medium group, consistent with balanced amino acid metabolism. Although palmitic acid and glycerol were interpreted as indicators of lipid mobilization, it is acknowledged that variations could also arise from dietary composition or rumen microbial metabolism. However, all diets shared comparable ingredient profiles and fat levels, minimizing the likelihood that these patterns were driven by compositional differences. The consistency of these metabolites across replicates and their alignment with known physiological mechanisms support the interpretation that the observed changes primarily reflect metabolic responses to nutrient supply rather than dietary artifacts. These patterns are comparable with biomarker studies in dairy cattle, where distinct amino acid, fatty acid, and carbohydrate profiles were associated with feed efficiency categories (Daddam *et al*., 2025; Giagnoni *et al*., 2025). Nevertheless, correlation alone is insufficient to establish functional prediction, and outcomes such as growth performance, stress resilience, or reproductive efficiency were beyond the scope of the present study.

The physiological relevance of these biomarker candidates was strengthened by correlation analyses. As shown in Figure 8, citric acid and D-fructose were strongly correlated with OMI and OMD in the high group, highlighting their role as indicators of efficient organic matter utilization. Nicotinic acid correlated most strongly with DMD, reflecting its role in redox regulation and energy metabolism. Threonine displayed consistent positive correlations with intake and digestibility in the medium group, supporting its role as a marker of balanced nutrient supply, while palmitic acid and glycerol in the low group reflected adaptive lipid mobilization under deficiency. Similar correlation patterns between metabolites and intake–digestibility measures were previously observed in cattle (Du *et al*., 2023) and sheep (Zhang *et al*., 2025b), suggesting that such relationships are conserved across ruminants. Although the sample size was limited, it is consistent with established practices in exploratory metabolomics, where multivariate modeling and internal cross-validation are used to extract biologically meaningful patterns from small but well-controlled datasets. The clear clustering observed in PCA and PLS-DA, along with consistent correlations between metabolites and nutritional parameters, suggests the presence of structured metabolic variation associated with nutritional status; however, these patterns should be interpreted cautiously and require validation in larger, independent cohorts to exclude random variation or model overfitting.

From a translational perspective, this work does not advocate the direct on-farm use of GC–MS technology but positions metabolomics as a discovery-level approach to elucidate metabolic pathways and identify candidate markers associated with nutritional status under tropical conditions. The translational pathway is conceptualized as stepwise, beginning with untargeted metabolomic discovery, followed by validation in larger and more diverse populations, and ultimately progressing toward the development of simplified targeted assays or proxy indicators suitable for integration into nutritional advisory frameworks. Such downstream outputs may include targeted metabolite panels, simplified biochemical indicators, or decision-support thresholds that inform ration formulation without requiring metabolomic analysis at the farm level. While the present findings provide mechanistic insight into nutrient utilization, their applicability is constrained by the limited sample size and controlled experimental conditions, underscoring the need for further validation under on-farm settings. Accordingly, the metabolites identified here are not intended for immediate practical application but serve to guide the selection of actionable indicators that may, upon validation, support evidence-based and context-appropriate precision feeding strategies in tropical goat production systems.

CONCLUSION

In conclusion, integrating intake–digestibility parameters with plasma metabolomic profiling provided a more integrative and informative framework for assessing nutritional status in Saanen goats under tropical conditions. Organic matter intake and digestibility appeared to be among the more responsive conventional indicators, while metabolomic analyses suggested the presence of diet-associated, group-specific metabolic patterns. Pathways related to carbohydrate, amino acid, and energy metabolism were preliminarily implicated, and six metabolites including citric acid, nicotinic acid, D-fructose, threonine, palmitic acid, and glycerol were preliminarily identified as potential biomarker candidates differentiating nutritional groups. Taken together, these findings suggest that metabolomics may complement conventional nutritional measures and contribute to the development of more informed precision feeding strategies for sustainable goat production in tropical environments. However, given the exploratory nature of this study and the limited sample size, further validation in larger, independent, and on-farm studies is required to confirm the robustness and applicability of these candidate biomarkers under variable tropical conditions.

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NOVELTY STATEMENT

This study integrates conventional feed intake and digestibility indices with plasma metabolomic profiling to characterize nutritional status in Saanen goats raised under tropical conditions. The findings demonstrate that metabolomic signatures capture diet-associated metabolic adaptations that are not fully reflected by dry matter–based nutritional indices alone, particularly under persistent tropical heat stress. The identification of organic matter–associated metabolite biomarkers (citric acid, nicotinic acid, D-fructose, threonine, palmitic acid, and glycerol) provides pathway-level insight into nutrient utilization efficiency, offering a mechanistic basis to support precision nutritional monitoring and feeding strategies in tropical dairy goat production systems.

AUTHORS CONTRIBUTION

FI, AMD, KDDA, IUA, ED, and MIA. conceived and designed the experiment. FI, IUA, ED, and SN performed the experimental procedures. FI, AMD, KDDA, IUA, ED, SN, and MIA supervised and coordinated the research and provided clinical data. Statistical analysis was conducted by AMD and SN. The initial draft of the manuscript was prepared by FI, AMD, KDDA, IUA, and ED. All authors equally contributed and approved the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interests.

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