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Comparative Metabolomic Analysis of Lactating Saanen Goats with Different Milk Production Levels

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ABSTRACT

Variation in milk yield among dairy goats maintained under identical feeding and management conditions suggests underlying differences in metabolic organization and nutrient utilization efficiency. This study aimed to evaluate the precision of nutritional status in lactating Saanen dairy goats classified by milk production level by integrating productive performance, energy metabolic biomarkers, hormonal profiling, and untargeted serum metabolomics. Twenty multiparous goats were categorized into high production (HP; n = 10) and low production (LP; n = 10) groups based on average daily milk yield. Circulating non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), insulin, cortisol, and insulin-like growth factor-1 (IGF-1) were measured, and serum metabolomic profiling was performed using LC–HRMS followed by multivariate and pathway enrichment analyses. HP goats produced significantly more milk (3.93 ± 0.35 L/day) than LP goats (2.55 ± 0.19 L/day) and exhibited lower NEFA, BHBA, and cortisol concentrations, indicating greater metabolic efficiency and reduced stress-associated burden. Untargeted metabolomics identified 54 annotated metabolites, of which 25 were significantly altered between groups. Multivariate analysis demonstrated clear metabolic separation, and pathway enrichment highlighted tryptophan metabolism, one-carbon metabolism, riboflavin metabolism, and carbohydrate-related pathways as key differentiating routes. HP goats were characterized by higher uridine diphosphate glucose, riboflavin, S-adenosylmethionine, and betaine, whereas LP goats showed increased kynurenine, L-formylkynurenine, 1-methylhistidine, cortisone, and methylmalonic acid. Nine metabolites exhibited strong discriminative performance (AUC > 0.8), supporting their potential as biomarker candidates. These findings indicate that milk production divergence in Saanen dairy goats reflects coordinated differences in systemic metabolic remodeling, defining distinct precision nutritional status phenotypes associated with metabolic efficiency and stress–energy adaptation.

Keywords: Saanen dairy goats; milk production; precision nutritional status; metabolic efficiency; metabolomics

INTRODUCTION

Saanen dairy goats are widely recognized as one of the most productive dairy goat breeds and contribute substantially to milk production systems at both national and global levels (Irawan *et al*., 2026). In intensive dairy goat operations, optimizing milk yield while maintaining metabolic stability is essential for ensuring production efficiency, economic sustainability, and animal welfare (Pardo *et al*., 2022). However, considerable inter-individual variation in milk production is frequently observed even among animals maintained under identical feeding and management conditions (Dosseh *et al*., 2025). Such variability suggests that productive performance is not solely determined by nutrient supply but is also influenced by differences in metabolic efficiency and physiological adaptability.

Lactation is an energy-demanding process that requires coordinated regulation of nutrient partitioning, endocrine signaling, and systemic metabolic adaptation (Gross *et al*., 2022). During high milk synthesis, glucose, fatty acids, and amino acids are preferentially allocated

to mammary gland activity, leading to dynamic changes in whole-body energy balance (Irawan *et al*., 2026). Circulating non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) are commonly used indicators of lipid mobilization and ketogenesis, whereas insulin, cortisol, and insulin-like growth factor-1 (IGF-1) reflect key aspects of glucose utilization, stress response, anabolic signaling, and mammary function (Zamuner *et al*., 2020a; Caputo *et al*., 2021; Zheng *et al*., 2025). Together, these metabolic and endocrine indicators provide useful insight into lactational energy status, although they may not fully capture the complexity of biochemical networks underlying individual differences in milk yield. Conventional assessment of nutritional status in dairy goats primarily relies on production records, body condition scoring, and selected biochemical indicators (Andjelić *et al*., 2022). While these approaches are valuable for herd-level management, they offer limited insight into systemic metabolic coordination at the individual-animal level (Ghavipanje *et al*., 2021). Increasing evidence suggests that productive variability is closely related to differences in metabolic resilience, nutrient-use efficiency, and pathway-level biochemical regulation (Islamiyati *et al*., 2025). Therefore, integrative molecular approaches are required to characterize better metabolic phenotypes associated with variations in milk production.

Metabolomics has emerged as a powerful analytical platform for profiling low-molecular-weight metabolites that represent the downstream products of gene expression and enzymatic activity (Yusuf *et al*., 2026). By providing a comprehensive snapshot of metabolic processes, metabolomic analysis enables the identification of pathway-level alterations associated with physiological adaptation and production efficiency. In ruminant research, metabolomic profiling has been increasingly applied to investigate energy balance, metabolic disorders, and lactational physiology (Irawan *et al*., 2026). Alterations in amino acid metabolism, lipid pathways, carbohydrate utilization, and oxidative stress-related metabolites have been implicated in variability in productive performance, highlighting the potential of metabolomics within precision nutrition frameworks (Carlino *et al*., 2025).

Despite growing interest in precision livestock management and metabolomic technologies, comparative characterization of systemic metabolic signatures between high- and low-producing dairy goats maintained under uniform management conditions remains limited (Islamiyati *et al*., 2025). In the present context, precise nutritional status is considered an integrative metabolic phenotype inferred from production performance, energy metabolic biomarkers, endocrine indicators, and serum metabolomic profiles, rather than a direct measurement of individual feed or nutrient intake. Identifying distinct metabolic and endocrine patterns associated with productive differences may enhance understanding of nutrient utilization efficiency and support the development of biomarker-based strategies for metabolite-informed nutritional evaluation.

Therefore, the present study aimed to evaluate and compare production-associated metabolic and metabolomic phenotypes in lactating Saanen dairy goats classified by milk production level. By integrating milk production performance, energy metabolic biomarkers (NEFA and BHBA), hormonal profiles (insulin, cortisol, and IGF-1), and LC–HRMS-based untargeted serum metabolomics, this study sought to identify differential serum metabolites and associated metabolic pathways related to variations in milk yield. The findings are expected to provide associative biological insights into metabolic efficiency and support the development of metabolite-informed nutritional evaluation strategies in dairy goats.

MATERIALS AND METHODS

Experimental Design and Ethical Approval

This study was conducted to evaluate the precision nutritional status of lactating Saanen dairy goats through an integrative assessment of productive performance, energy metabolic profile, hormonal profile, and metabolomic signatures. A total of 20 multiparous lactating Saanen goats, defined as goats in their second to fourth parity, were enrolled and classified into two groups based on average daily milk yield recorded over a 14-day pre-experimental observation period: High Production (HP, n = 10) and Low Production (LP, n = 10). Goats with milk yield above the herd mean were assigned to the HP group, whereas those below the herd mean were assigned to the LP group.

All animals were maintained under identical housing and feeding management systems throughout the experimental period. The diet was formulated to meet the nutrient requirements of lactating dairy goats in accordance with NRC recommendations, and feed and water were provided ad libitum. To minimize confounding variation, animals were selected based on similar parity, body condition score (BCS), and stage of lactation. Within the precision nutrition framework, milk production was considered a functional phenotypic indicator of nutrient utilization efficiency and metabolic adaptation. Therefore, productive, metabolic, hormonal, and metabolomic parameters were integratively evaluated to characterize individual nutritional status.

All experimental procedures involving animal handling and biological sampling were conducted in accordance with institutional ethical standards and were approved by the Ethics Committee of the Department of Animal Sciences, Faculty of Agriculture, Lambung Mangkurat University, Banjarbaru, Indonesia (Approval No. 037/UN1.23.5/PG/2026).

Milk Production Measurement

Milk production was measured individually throughout the study period using standardized milking and recording procedures (Landi *et al*., 2021). All Saanen goats were milked twice daily (morning and afternoon) at consistent times to minimize diurnal variation. Milk from each session was collected in calibrated containers and immediately quantified using a graduated cylinder or volumetric milk meter. Milk

volume was recorded in liters (L), and daily milk yield (L/day) was calculated as the sum of morning and afternoon production for each animal.

Milk yield was recorded for 14 consecutive days prior to biological sampling to obtain representative baseline production data. Recording was continued during the sampling period to ensure accurate alignment of productive performance with metabolic, hormonal, and metabolomic analyses.

Blood Sampling and Serum Preparation

Blood samples were collected from the jugular vein of each goat prior to the morning feeding to minimize postprandial variation. Approximately 5 mL of blood was drawn into plain vacuum tubes without anticoagulant and allowed to clot at room temperature for 20–30 minutes. Samples were centrifuged at 3,000 rpm for 15 minutes to separate serum (Nurlatifah *et al*., 2025). The obtained serum was aliquoted into sterile microtubes and stored at −80 °C until further biochemical, hormonal, and metabolomic analyses.

Energy Metabolic Profile

To evaluate systemic energy balance and metabolic adaptation, circulating non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) were measured as indicators of the energy metabolic profile. Serum NEFA concentrations were determined using a commercial enzymatic colorimetric assay kit (NEFA Assay Kit, Randox Laboratories Ltd., Crumlin, United Kingdom), while BHBA concentrations were measured using a β-Hydroxybutyrate Assay Kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer's instructions (Barletta *et al*., 2017; Benedet *et al*., 2020). Absorbance readings were obtained using a microplate spectrophotometer at the specified wavelength, and metabolite concentrations were calculated based on standard calibration curves provided with the kits. Results were expressed in mmol/L.

Hormone Profile

Hormonal profiling was performed to evaluate endocrine responses associated with nutrient utilization, metabolic regulation, and lactational performance. Serum insulin, cortisol, and insulin-like growth factor-1 (IGF-1) concentrations were determined using commercially available goat-specific enzyme-linked immunosorbent assay (ELISA) kits (Goat Insulin ELISA Kit, MyBioSource Inc., San Diego, CA, USA; Goat Cortisol ELISA Kit, Elabscience Biotechnology Co., Ltd., Wuhan, China; Goat IGF-1 ELISA Kit, MyBioSource Inc., San Diego, CA, USA), following the manufacturers' instructions (Zamuner *et al*., 2020b; Santoso *et al*., 2024). Briefly, serum samples and standards were added to antibody-coated microplate wells and incubated according to the recommended protocol. After appropriate washing steps and substrate reaction, the optical density was measured at 450 nm using a microplate reader. Hormone concentrations were calculated based on standard calibration curves generated from known standards and expressed in ng/mL.

Metabolomic Analysis

Untargeted metabolomic analysis of serum samples from lactating Saanen dairy goats was performed using a liquid chromatography–high resolution mass spectrometry (LC–HRMS) platform following the procedure of Islamiyati *et al*. (2025). Serum samples obtained as described previously were subjected to metabolite extraction using a methanol-based protein precipitation method. Briefly, 100 µL of serum was transferred into Eppendorf tubes and mixed with prechilled 80% methanol by vigorous vortexing. Samples were incubated on ice for 5 min and centrifuged at 15,000 × g for 20 min at 4 °C. The supernatant was collected, diluted with LC–MS-grade water to obtain a final methanol concentration of 53%, transferred into a fresh tube, and centrifuged again at 15,000 × g for 20 min at 4 °C to remove residual particulates. The resulting supernatant was subjected to LC–MS/MS analysis.

Chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC system equipped with a binary pump and an Accucore™ C18 column (100 mm × 2.1 mm ID, 2.6 µm) maintained at 40 °C. The mobile phases consisted of MS-grade water with 0.1% formic acid (A) and MS-grade acetonitrile with 0.1% formic acid (B). The flow rate was set at 0.3 mL/min with a total run time of 25 min. The gradient elution program was as follows: 5% B at initial conditions, increased to 90% B over 16 min, held at 90% B for 4 min, and returned to 5% B for column re-equilibration until 25 min. The injection volume was 5 µL.

Mass spectrometric detection was carried out using a Thermo Scientific™ Orbitrap™ Exploris 240 HRMS operated in Full MS/data-dependent MS2 (dd-MS2) acquisition mode with polarity switching in both positive and negative ionization modes. Full MS scans were acquired at 60,000 FWHM resolution over an m/z range of 67–1000 with a mass accuracy threshold of 5 ppm. Data-dependent MS2 spectra were obtained at 30,000 FWHM using stepped normalized collision energies of 30, 50, and 70.

Raw LC–MS data were processed using Thermo Scientific™ Compound Discoverer™ 3.5 for peak detection, alignment, background subtraction, and normalization. Metabolite annotation was performed by matching MS/MS spectra against the mzCloud library. Structural prediction was further supported by SIRIUS using the KEGG and PubChem databases. Additional annotation was conducted through ChemSpider-integrated resources, including the Human Metabolome Database (HMDB), KEGG, PubChem, ChEBI, ChEMBL, FooDB, and species-relevant metabolome databases. Mass list searches were also performed using HMDB v5 and the Natural Products Atlas (2024_09 release).

Bioinformatic and Statistical Analysis

The processed feature table from Thermo Scientific™ Compound Discoverer™ 3.5 was used for

downstream analysis. Metabolites were filtered based on annotation confidence, MS/MS quality, and mass accuracy (≤ 5 ppm), verified using mzCloud, and cross-referenced with KEGG and PubChem. Only reliably identified metabolites were retained and reported using KEGG compound IDs. Data were log-transformed and Pareto-scaled prior to multivariate analysis. PCA and PLS-DA were performed using MetaboAnalyst 6.0 to assess clustering and identify discriminative metabolites between HP and LP groups. The performance of the PLS-DA model was evaluated using R^2 and Q^2 values, where R^2 indicates the goodness of fit and Q^2 represents predictive ability estimated through cross-validation. To assess model robustness and reduce the risk of overfitting, permutation testing was performed by randomly permuting class labels and comparing the resulting model statistics with those of the original model. Differential metabolites were identified using volcano plot analysis with independent-samples t-tests (or Mann–Whitney U tests when appropriate), applying thresholds of $p<0.05$ and VIP > 1.0, with FDR correction. Hierarchical clustering and KEGG-based pathway enrichment analyses were conducted within MetaboAnalyst.

Productive, energy metabolic, and hormonal parameters were analyzed using IBM SPSS Statistics version 26. Normality was evaluated using the Shapiro–Wilk test, and appropriate parametric or non-parametric tests were applied. Results were expressed as mean ± SD, with $p<0.05$ considered significant. Pearson correlation analysis was used to assess associations between physiological parameters (milk yield, NEFA, BHBA, insulin, cortisol, and IGF-1) and selected biomarker candidate metabolites, with significance set at $p<0.05$.

RESULTS

Milk Production Performance

Daily milk yield differed markedly between the two production groups (Figure 1). Goats classified in the high production (HP) group produced significantly greater milk volume compared to those in the low production (LP) group (HP: 3.93 ± 0.35 L/day vs. LP: 2.55 ± 0.19 L/day; $p<0.05$). The HP group consistently exhibited higher individual production values and a wider distribution, whereas LP goats showed lower and more homogeneous milk output. The clear separation between groups confirmed a distinct productive divergence under identical management and feeding conditions.

Energy Metabolic Profile

Energy metabolic profile parameters differed significantly between production groups (Figure 2). NEFA concentrations were significantly lower in the HP group compared to the LP group (HP: 0.256 ± 0.028 mmol/L vs. LP: 0.470 ± 0.061 mmol/L; $p<0.05$). Similarly, BHBA concentrations were significantly reduced in HP goats relative to LP goats (HP: 0.684 ± 0.081 mmol/L vs. LP: 1.115 ± 0.118 mmol/L; $p<0.05$). Overall, LP goats exhibited markedly elevated circulating NEFA and BHBA concentrations compared to HP goats.

Hormonal Profile

Hormonal profiles in HP and LP goats are summarized in Table 1. No significant differences were observed in IGF-1 and insulin levels between the two production groups ($p>0.05$). Both parameters showed comparable distributions across HP and LP goats, indicating similar anabolic and metabolic regulatory status between groups. In contrast, cortisol concentrations differed significantly between groups ($p<0.05$), with LP goats exhibiting higher levels compared to HP goats. The separation in cortisol values between groups was consistent across individual animals, indicating a clear difference in stress-associated endocrine responses by to production level.

Distribution of Identified Metabolites

After quality control filtering and annotation verification, a total of 54 serum metabolites with confirmed

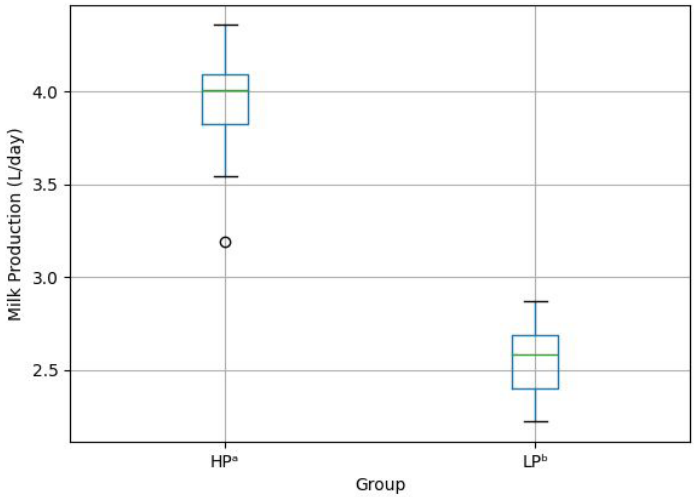

Figure 1. Daily milk production (L/day) in high production (HP) and low production (LP) Saanen dairy goats. Different superscript letters indicate significant differences between groups ($p<0.05$).

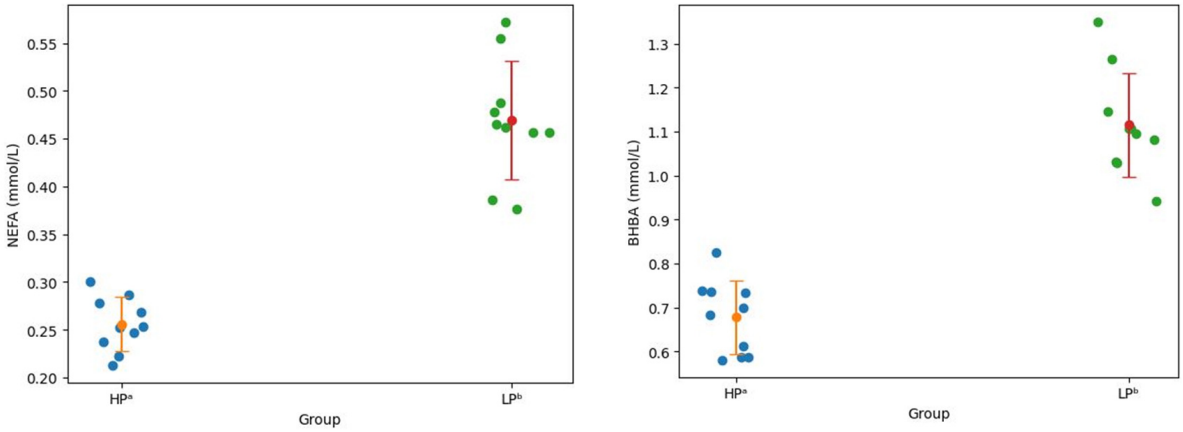

Figure 2. Energy metabolic profile in high production (HP) and low production (LP) Saanen dairy goats. (A) Non-esterified fatty acids (NEFA) concentrations. (B) Beta-hydroxybutyrate (BHBA) concentrations. Different superscript letters indicate significant differences between groups ($p<0.05$).

Table 1. Hormonal profile in high production (HP) and low production (LP) Saanen dairy goats

Parameter	Mean ± SD		p-value
	HP	LP	
IGF-1 (ng/mL)	302.90 ± 18.53	289.26 ± 42.83	0.368
Insulin (ng/mL)	16.11 ± 2.71	15.06 ± 4.17	0.513
Cortisol (ng/mL)	18.20 ± 4.58^{b}	30.83 ± 4.46^{a}	<0.001

Note: Different superscript letters within the same row indicate significant differences between groups ($p<0.05$).

KEGG compound IDs were retained for downstream analysis (Figure 3). Among these, 30 metabolites (55.6%) were commonly detected in the serum of both HP and LP goats. In contrast, 12 metabolites (22.2%) were uniquely identified in the serum of HP goats, and 12 metabolites (22.2%) were uniquely identified in the serum of LP goats. These results indicate a substantial overlap in core serum metabolic components between groups while also revealing distinct subsets of serum metabolites associated with production level.

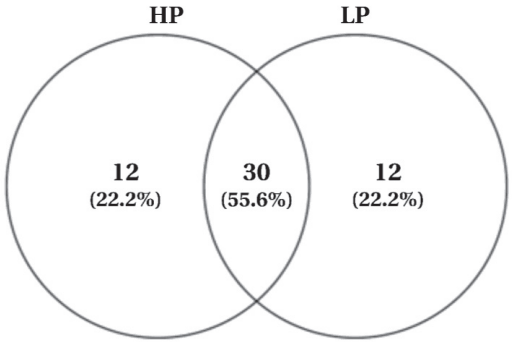

Figure 3. Venn diagram showing the distribution of serum metabolites retained after quality control filtering and KEGG-based annotation in high production (HP) and low production (LP) Saanen dairy goats.

Multivariate Analysis

Principal Component Analysis (PCA) was performed to explore global metabolic variation between groups (Figure 4A). The PCA score plot showed a clear separation between HP and LP goats primarily along PC1, which explained 49.5% of the total variance, with PC2 accounting for 8.1%. Together, the first two principal components explained 57.6% of the overall variance, indicating distinct metabolic profiles associated with production level. To further evaluate supervised discrimination, Partial Least Squares–Discriminant Analysis (PLS-DA) was conducted (Figure 4B). The PLS-DA model showed a clear separation between HP and LP groups, confirming distinct metabolic signatures. Several KEGG-annotated metabolites contributed strongly to group discrimination, as indicated by loading vectors in the biplot. Model performance was assessed using cross-validation (Figure 4C). The cross-validation and permutation testing supported the stability of the PLS-DA model. However, given the limited sample size, these results should be interpreted with caution as exploratory evidence of serum metabolomic separation between groups rather than definitive classification.

Differential Metabolites

Volcano plot analysis identified 25 metabolites with confirmed KEGG compound IDs that were significantly altered between HP and LP goats ($p<0.05$) (Figure 5A; Table 2). Among these, 13 metabolites were upregulated in HP goats, whereas 12 metabolites were upregulated in LP goats, indicating bidirectional metabolic shifts associated with production level. Several metabolites exhibited substantial $\log_2$ fold change values, accompanied by highly significant p-values, demonstrating strong statistical differentiation between groups.

To further identify metabolites contributing to supervised group discrimination, VIP analysis derived from the PLS-DA model was performed (Figure 5B). Among the 25 significant metabolites, 15 KEGG-annotated metabolites exhibited VIP scores exceeding the predefined threshold. These included C00029, C02305, C00328, C00255, C00207, C01152, C00019, C00378, C05984, C00762, C05635, C00719, C02477,

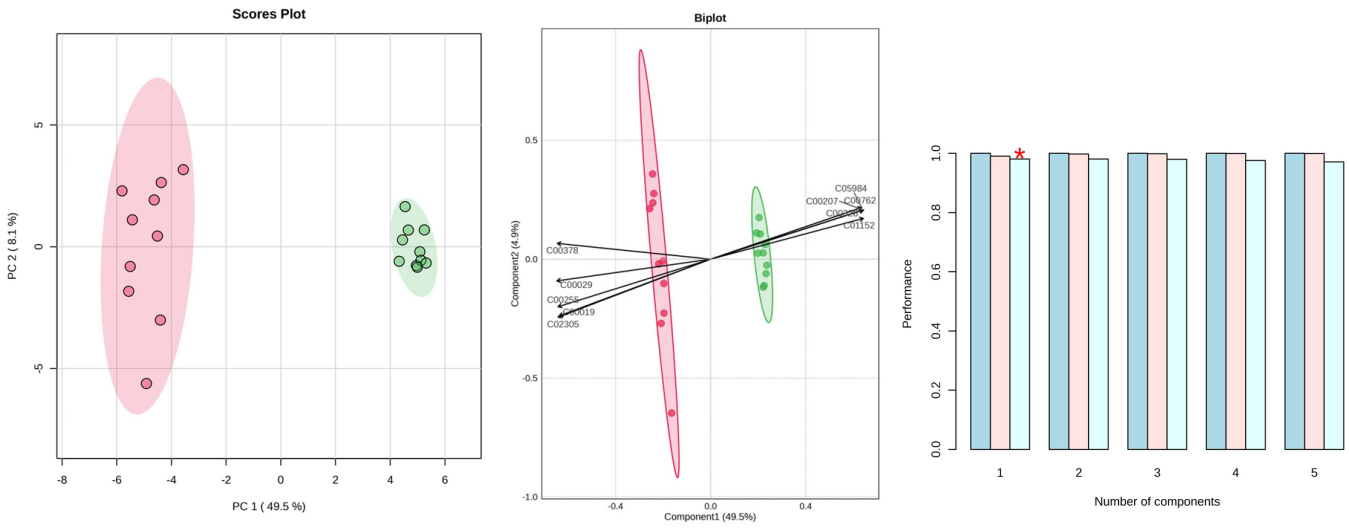

Figure 4. Multivariate analysis of QC-filtered KEGG-annotated metabolites in high production (HP) and low production (LP) Saanen dairy goats. (A) Principal component analysis (PCA) score plot; (B) PLS-DA biplot showing sample clustering and metabolite loadings; and (C) Cross-validation performance of the PLS-DA model across increasing numbers of components. Note : A= ○ HP, ○ LP; B= ■ HP, ■ LP; C= ■ Accuracy, ■ R2, ■ Q2

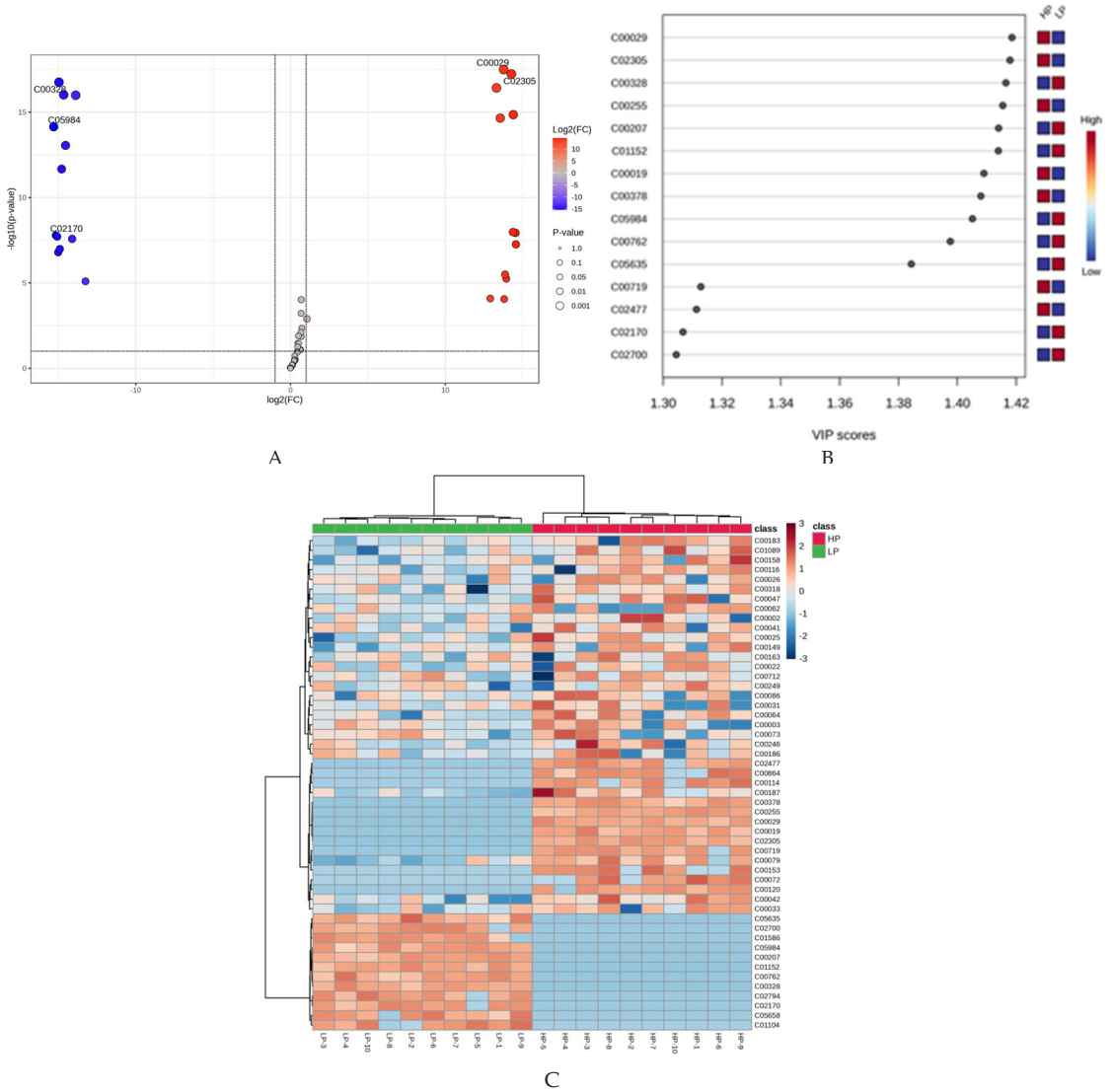

Figure 5. Identification of differential metabolites between high production (HP) and low production (LP) Saanen dairy goats. (A) Volcano plot showing significantly altered metabolites; (B) Variable Importance in Projection (VIP) scores from PLS-DA highlighting key discriminatory metabolites; and (C) Hierarchical clustering heatmap of significantly altered metabolites.

C02170, and C02700. These metabolites represented the most influential variables driving separation between HP and LP goats in the multivariate model.

Hierarchical clustering analysis of the significantly altered metabolites revealed distinct grouping patterns by production level (Figure 5C). HP and LP goats formed clearly separated clusters, with consistent intra-group similarity and marked inter-group divergence. The heatmap visualization revealed contrasting metabolite abundance patterns between groups, further supporting the metabolic differentiation identified through univariate and multivariate analyses.

Functional Pathway Enrichment Analysis

Pathway enrichment analysis identified 11 metabolic pathways associated with the differential metabolites that were detected with measurable pathway impact values, reflecting their topological involvement within the reconstructed metabolic network (Figure 6; Table 3). The identified pathways and their corresponding impact values were as follows: riboflavin metabolism (0.5), tryptophan metabolism (0.21368), one carbon pool by folate (0.13393), pentose and glucuronate interconversions (0.06024), cysteine and methionine metabolism (0.05271), amino sugar and nucleotide sugar metabolism (0.04888), glycine, serine and threonine metabolism (0.04766), valine, leucine and isoleucine degradation (0.02264), starch and sucrose metabolism (0.00974), steroid hormone biosynthesis (0.00663), and galactose metabolism (0.00228).

These pathways collectively represent the metabolic routes associated with the differential metabolite set identified between HP and LP goats.

Table 2. Differential metabolites in high production (HP) and low production (LP) Saanen dairy goats

KEGG ID	Metabolite	FC	log2(FC)	raw.pval	-log10(p)
C00029	Uridine diphosphate glucose (UDP-glucose)	13934	13.766	3.21E-18	17.493
C02305	Phosphocreatine	19708	14.267	5.84E-18	17.234
C00328	Kynurenine	3.13E-5	-14.962	1.77E-17	16.751
C00255	Riboflavin	10103	13.302	3.80E-17	16.421
C00207	Xanthine	3.87E-5	-14.656	9.63E-17	16.016
C01152	1-Methylhistidine	6.64E-5	-13.879	1.02E-16	15.992
C00019	S-Adenosyl-L-methionine (SAM)	21443	14.388	1.40E-15	14.853
C00378	Thiamine	11988	13.549	2.23E-15	14.652
C05984	2-Hydroxybutyric acid	2.48E-5	-15.299	7.20E-15	14.143
C00762	Cortisone	4.21E-5	-14.536	8.81E-14	13.055
C05635	5-Hydroxyindoleacetate	3.53E-5	-14.79	2.14E-12	11.669
C00719	Betaine	21145	14.368	1.05E-8	79.784
C02477	α-Tocopherol	23698	14.533	1.17E-8	79.302
C02170	Methylmalonic acid	2.73E-5	-15.16	1.64E-8	77.853
C02700	L-Formylkynurenine	2.89E-5	-15.077	1.93E-8	7.715
C02794	3-Hydroxykynurenine	5.65E-5	-14.111	2.64E-8	7.579
C00120	Biotin	24129	14.558	5.51E-8	72.588
C01586	Hippuric acid	3.26E-5	-14.903	1.03E-7	69.873
C05658	Indoxyl	3.03E-5	-15.011	1.61E-7	67.933
C00114	Choline	14837	13.857	3.18E-6	54.971
C00864	Pantothenic acid	15659	13.935	5.60E-6	52.518
C01104	Trimethylamine N-oxide	0.000	-13.246	8.03E-6	50.953
C00153	Nicotinamide	7661	12.903	8.17E-5	40.876
C00072	Ascorbate (L-ascorbic acid)	14264	13.8	8.74E-5	40.583
C00042	Succinate	20.799	10.565	0.001	28.986

Table 3. Pathway enrichment of differential metabolites identified between high production (HP) and low production (LP) Saanen dairy goats

Pathway name	p	-log(p)	Holm p	FDR	Impact	KEGG ID
Tryptophan metabolism	0.0040377	2.3939	0.32302	0.32302	0.21368	C01152; C02700; C00328
One carbon pool by folate	0.018686	1.7285	1	0.6911	0.13393	C00019; C00719
Riboflavin metabolism	0.033013	1.4813	1	0.6911	0.5	C00255
Starch and sucrose metabolism	0.1408	0.8514	1	1	0.00974	C00029
Pentose and glucuronate interconversions	0.14806	0.82957	1	1	0.06024	C00029
Galactose metabolism	0.20411	0.69013	1	1	0.00228	C00029
Cysteine and methionine metabolism	0.2439	0.61279	1	1	0.05271	C00019
Glycine, serine and threonine metabolism	0.25035	0.60145	1	1	0.04766	C00719
Valine, leucine and isoleucine degradation	0.28799	0.54062	1	1	0.02264	C02170
Amino sugar and nucleotide sugar metabolism	0.30015	0.52266	1	1	0.04888	C00029
Steroid hormone biosynthesis	0.48869	0.31096	1	1	0.00663	C00762

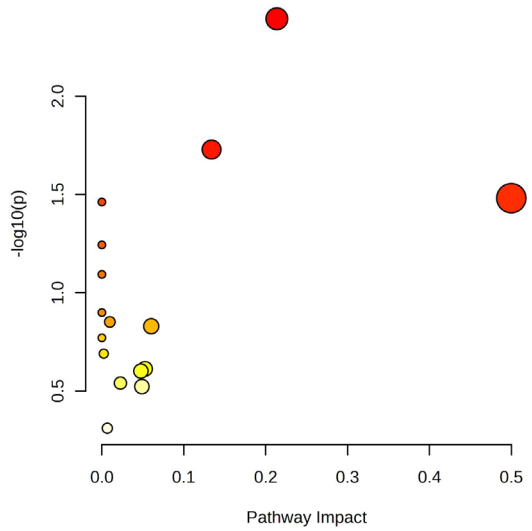

Figure 6. Pathway enrichment of differential metabolites.

Biomarker Candidate Performance

Based on the integrated metabolomic analysis, nine metabolites were identified as potential biomarker candidates capable of discriminating HP and LP groups. These candidates met all predefined criteria, including clear group-associated differential expression, a VIP score > 1.0 in the PLS-DA model, involvement in pathways identified in the enrichment analysis, and strong discriminative performance with an AUC > 0.8. The detailed information regarding KEGG ID, metabolite name, associated group, VIP score, related pathway, and AUC values is presented in Table 4. The selected metabolites comprised C00029 (uridine diphosphate glucose), C00328 (kynurenine), C00255 (riboflavin), C01152 (1-methylhistidine), C00019 (S-adenosylmethionine), C00762 (cortisone), C00719 (betaine), C02170 (methylmalonic acid), and C02700 (L-formylkynurenine).

Several metabolites were predominantly associated with the HP group, including uridine diphosphate glucose (C00029), riboflavin (C00255), S-adenosylmethionine (C00019), and betaine (C00719), whereas kynurenine (C00328), 1-methylhistidine (C01152), cortisone (C00762), methylmalonic acid (C02170), and L-formylkynurenine (C02700) were associated with the LP group. Notably, riboflavin, S-adenosylmethionine, betaine, methylmalonic acid, and L-formylkynurenine demonstrated perfect discrimination performance (AUC = 1.00), while the remaining metabolites also exhibited excellent classification ability (AUC range 0.93–0.98), as summarized in Table 4.

As illustrated in Figure 7, the boxplot clearly demonstrates distinct separation between HP and LP groups for each candidate metabolite. HP-associated metabolites showed consistently higher relative intensities in the HP group and lower levels in LP, whereas LP-associated metabolites exhibited the opposite pattern. Minimal overlap between groups further supports the robustness of these features as discriminative features. The consistency between multivariate importance (VIP), pathway involvement, and ROC performance strengthens the reliability of these metabolites as potential indicators of metabolic divergence between HP and LP.

Correlation Analysis

Correlation analysis was conducted to examine the associations between physiological parameters and selected biomarker candidate metabolites within each production group (Figure 8A–B). Only statistically significant correlations are described. In the HP group (Figure 8A), riboflavin (C00255) showed significant positive correlations with milk production and NEFA. S-adenosylmethionine (C00019) was significantly and positively correlated with BHBA. No other significant associations were observed in the HP group. In the LP group (Figure 8B), kynurenine (C00328) demonstrated a significant positive correlation with IGF-1. 1-Methylhistidine (C01152) showed significant negative correlations with IGF-1 and insulin. Cortisone (C00762) exhibited a significant and strong positive correlation with BHBA. Overall, significant correlations in the HP group were primarily associated with production performance and lipid mobilization parameters, whereas in the LP group, significant associations were mainly related to endocrine regulation and ketone body metabolism.

DISCUSSION

This study demonstrates that variation in milk yield among lactating Saanen dairy goats maintained under similar management conditions was associated

Table 4. ROC performance of selected VIP metabolites identified as potential biomarker candidates distinguishing high production (HP) and low production (LP) Saanen dairy goats

KEGG ID	Metabolite	Group	VIP	Pathway	AUC
C00029	Uridine diphosphate glucose	HP	1,41858	4	0,93
C00328	Kynurenine	LP	1,41651	1	0,96
C00255	Riboflavin	HP	1,41546	1	1,00
C01152	1-Methylhistidine	LP	1,41395	1	0,98
C00019	S-Adenosylmethionine	HP	1,40904	2	1,00
C00762	Cortisone	LP	1,39762	1	0,94
C00719	Betaine	HP	1,31281	2	1,00
C02170	Methylmalonic acid	LP	1,30675	1	1,00
C02700	L-Formylkynurenine	LP	1,30446	1	1,00

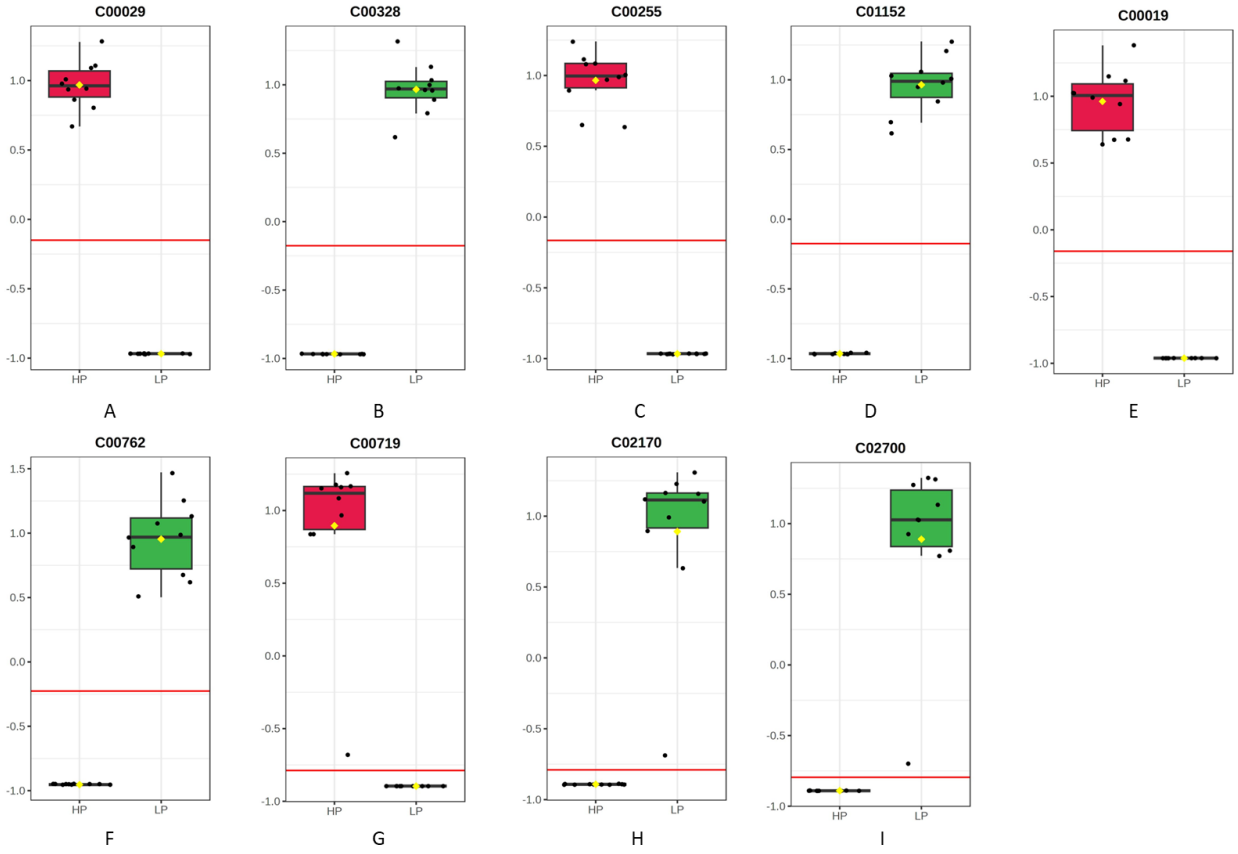

Figure 7. Boxplot distributions of selected biomarker candidate metabolites discriminating high production (HP) and low production (LP) Saanen dairy goats. (A) S-adenosylmethionine, (B) uridine diphosphate glucose, (C) riboflavin, (D) kynurenine, (E) betaine, (F) cortisone, (G) 1-methylhistidine, (H) methylmalonic acid, and (I) L-formylkynurenine.

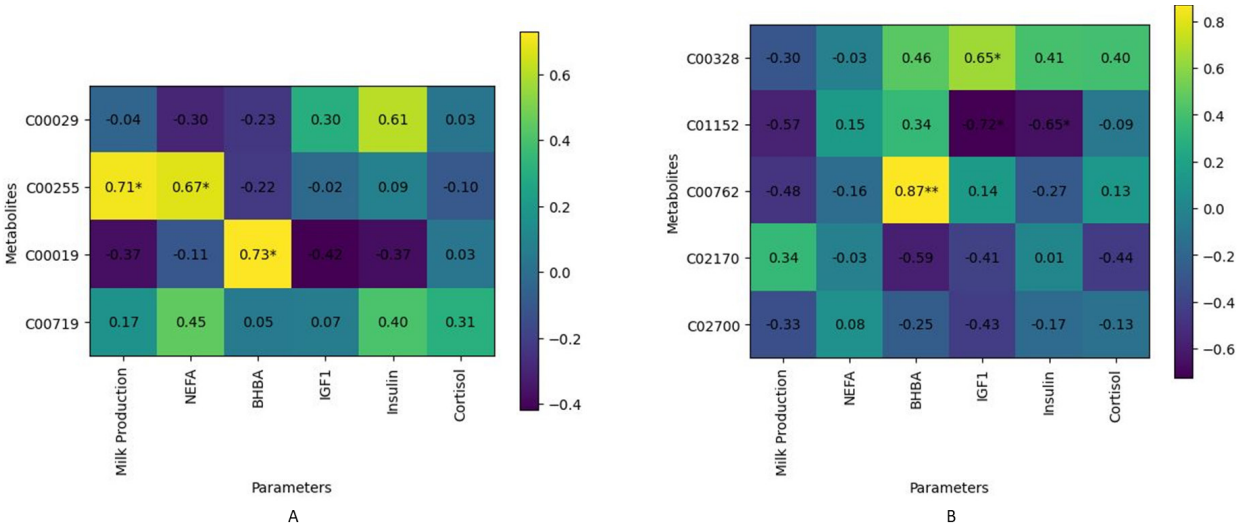

Figure 8. Pearson correlation heatmaps showing the relationships between physiological parameters and selected biomarker candidate metabolites in Saanen dairy goats. (A) High production (HP) group and (B) low production (LP) group. Asterisks indicate statistically significant correlations ($p<0.05$). C00029= Uridine diphosphate glucose; C00328= Kynurenine; C00255= Riboflavin; C01152= 1-Methylhistidine; C00019= S-Adenosylmethionine; C00762= Cortisone; C00719= Betaine; C02170= Methylmalonic acid; and C02700= L-Formylkynurenine.

with coordinated differences in energy metabolism, endocrine response, and serum metabolomic profiles. Although more than half of the annotated serum metabolites were shared between HP and LP goats, multivariate analysis, differential metabolite testing, and pathway enrichment consistently indicated distinct serum metabolic patterns between production groups (Islamiyati *et al.*, 2025). The main pathways involved included tryptophan-related immunometabolic signaling, one-carbon metabolism, riboflavin-linked redox regulation, carbohydrate-related metabolism, and organic acid metabolism (Lima *et al.*, 2022; Daddam *et*

al., 2025). These findings suggest that differences in milk production level were associated with systemic metabolic variation rather than isolated changes in individual metabolites (Wang *et al*., 2024).

A central physiological difference between the production groups was observed in the energy metabolic profile. LP goats exhibited markedly higher NEFA and BHBA concentrations, indicating greater lipid mobilization and ketogenesis, which are commonly associated with a more pronounced negative energy balance during lactation (Ghavipanje *et al*., 2021; Lisuzzo *et al*., 2022). In contrast, HP goats showed lower NEFA and BHBA despite producing more milk, suggesting that higher milk output was associated with a less pronounced lipid mobilization and ketogenesis response. This finding indicates a more stable energy metabolic profile in HP goats than in LP goats and is consistent with previous reports linking metabolic stability and oxidative balance to production performance in dairy animals (Meli *et al*., 2025; Ponnampalam *et al*., 2024).

Endocrine profiling showed that LP goats had significantly higher cortisol concentrations, whereas IGF-1 and insulin did not differ significantly between groups. This finding indicates that the difference between HP and LP goats was more evident in stress-associated endocrine response than in anabolic hormone status. Cortisol is closely related to hypothalamic–pituitary–adrenal axis activity and may influence glucose metabolism, lipid mobilization, and stress adaptation (Knezevic *et al*., 2023; Ünal & Uztimür, 2025). The detection of cortisone as an LP-associated discriminatory metabolite further supports the involvement of glucocorticoid-related metabolism in the LP phenotype. In addition, the positive correlation between cortisone and BHBA in LP goats suggests an association between glucocorticoid-related metabolism and ketone body production, although this relationship should be interpreted as correlative rather than causal (Zamuner *et al*., 2020b).

At the pathway level, LP goats showed higher kynurenine and L-formylkynurenine, indicating that tryptophan metabolism differed between production groups. Kynurenine pathway activity has been associated with immune activation, oxidative stress adaptation, and endocrine–metabolic interactions (Tsuji *et al*., 2023; Sorboni *et al*., 2022). In the present study, the increase in kynurenine-related metabolites in LP goats was accompanied by higher cortisol, NEFA, and BHBA concentrations, suggesting that this pathway may be associated with the stress–energy metabolic profile observed in the LP group. The positive correlation between kynurenine and IGF-1 in LP goats further suggests a possible link between tryptophan metabolism and endocrine regulation, although this association requires further validation (Salama *et al*., 2021; Chen *et al*., 2025).

LP goats also exhibited higher 1-methylhistidine, which may reflect increased protein turnover or dietary muscle-derived compounds. Under conditions of increased energy demand or metabolic challenge, protein mobilization may contribute to the supply of energy and glucose precursors in lactating animals (Siachos *et al*., 2024). In this study, 1-methylhistidine was negatively correlated with IGF-1 and insulin in LP goats, suggesting an association between this metabolite and reduced anabolic endocrine signaling. Together with higher NEFA, BHBA, and cortisol, this finding supports the interpretation that LP goats had a more catabolic metabolic profile than HP goats (Li *et al*., 2026).

In contrast to LP goats, HP goats were characterized by higher UDP-glucose, S-adenosylmethionine, betaine, and riboflavin. UDP-glucose is related to carbohydrate activation and glycosylation pathways, and carbohydrate utilization is particularly important during lactation because glucose supports lactose synthesis and milk volume regulation (Lin *et al*., 2016). In the present study, higher UDP-glucose in HP goats, together with lower NEFA and BHBA concentrations, suggests that the HP phenotype was associated with a more stable systemic energy profile and less dependence on lipid mobilization (Zamuner *et al*., 2020b).

HP goats also showed higher S-adenosylmethionine and betaine, both of which are involved in one-carbon metabolism and methyl-group transfer reactions (Jiang *et al*., 2023). One-carbon metabolism is linked with methylation processes, hepatic metabolic function, antioxidant defense, and metabolic adaptation (Clare *et al*., 2019). In this study, higher S-adenosylmethionine and betaine in HP goats may indicate differences in methyl-donor-related metabolism between production groups. The positive correlation between S-adenosylmethionine and BHBA in the HP group may also suggest an adaptive association between methyl-donor metabolism and lipid-derived energy use, although this interpretation should be approached with caution, given the limited sample size (Ghavipanje *et al*., 2021).

Riboflavin emerged as another prominent HP-associated metabolite with high pathway relevance and discriminative performance. Riboflavin is a precursor of flavin cofactors involved in mitochondrial oxidative metabolism, fatty acid oxidation, and redox balance (Hampel *et al*., 2016; Ponnampalam *et al*., 2024). In the present study, higher riboflavin levels in HP goats and their positive correlation with milk production suggest that riboflavin metabolism is associated with the high-production phenotype. This finding is consistent with the lower NEFA, BHBA, and cortisol levels observed in HP goats and may reflect a more favorable metabolic condition for sustaining milk production, although targeted validation is still required (Meli *et al*., 2025).

Beyond these core pathways, enrichment analysis also identified carbohydrate- and amino acid-related pathways, as well as steroid hormone biosynthesis. Although some pathways had modest impact values, their collective appearance suggests that production-level differences were associated with coordinated changes in substrate metabolism and endocrine-related pathways (Ni *et al*., 2025; Zamuner *et al*., 2020b). The steroid hormone biosynthesis signal was consistent with the observed differences in cortisol and cortisone between groups, supporting an association between endocrine response and serum metabolomic variation in LP goats (Ünal & Uztimür, 2025).

The integration of VIP scores, univariate significance, pathway involvement, and ROC analysis identified

nine candidate metabolites that differentiated HP and LP goats. HP-associated metabolites included UDP-glucose, riboflavin, S-adenosylmethionine, and betaine, whereas LP-associated metabolites included kynurenine, L-formylkynurenine, 1-methylhistidine, cortisone, and methylmalonic acid. This integrated approach is commonly used to prioritize candidate biomarkers in metabolomics studies, although such candidates require further validation before practical application (Cardoso *et al*., 2024). Methylmalonic acid was particularly relevant in LP goats because it may reflect altered organic acid metabolism and vitamin B12-related metabolic function, which can influence energy metabolism in ruminants (Liu *et al*., 2022). Therefore, the LP-associated metabolite profile may indicate greater stress-associated and catabolic metabolic adaptation, whereas the HP-associated profile may indicate more stable substrate and cofactor-related metabolism (Salama *et al*., 2021; Tsuji *et al*., 2023).

From a translational perspective, the present findings suggest that serum metabolomics can complement conventional indicators such as milk yield, NEFA, BHBA, and hormone profiles by providing additional pathway-level information on production-associated metabolic phenotypes (Ghavipanje *et al*., 2021; Lisuzzo *et al*., 2022). A composite panel comprising UDP-glucose, riboflavin, S-adenosylmethionine, betaine, kynurenine pathway metabolites, cortisone, 1-methylhistidine, and methylmalonic acid may serve as a candidate indicator panel for identifying goats with distinct metabolic profiles under intensive management. However, because individual feed intake was not measured and the sample size was limited, the practical application of this panel for precision nutrition should be considered preliminary and requires further validation (Clare *et al*., 2019; Pardo *et al*., 2022).

The correlation analysis further supported the physiological relevance of selected biomarker candidates. In HP goats, riboflavin was positively correlated with milk production, while S-adenosylmethionine was positively correlated with BHBA. In LP goats, kynurenine was positively correlated with IGF-1, 1-methylhistidine was negatively correlated with IGF-1 and insulin, and cortisone was positively correlated with BHBA. These findings suggest that the candidate metabolites were associated with production, endocrine, and energy metabolic parameters. However, these correlations do not imply causality and should be interpreted cautiously.

Several limitations should be acknowledged. First, individual feed intake and nutrient intake were not measured in this study. Therefore, the concept of precision nutritional status should be interpreted as an integrative metabolic and metabolomic phenotype under similar feeding and management conditions, rather than as a direct measurement of individual nutrient consumption. Second, the cohort size was relatively small (n = 10 per group), which may inflate apparent classification performance, increase the risk of overfitting in multivariate analyses such as PLS-DA, and limit generalizability across farms, diets, and lactation stages. Therefore, the multivariate classification results should be interpreted as exploratory and should be validated in larger independent datasets. Third, untargeted metabolomics provides relative abundance rather than absolute quantification; thus, targeted validation with standardized assays is necessary before defining actionable thresholds (Islamiyati *et al*., 2025). Fourth, serum reflects systemic metabolism and cannot directly specify tissue-level flux in the liver, adipose tissue, or mammary gland (Sun *et al*., 2017). Future studies should incorporate individual feed intake and nutrient intake measurements, larger multi-farm populations, longitudinal sampling across lactation phases, targeted quantification of the key metabolites identified, and integration with inflammatory markers and rumen-derived energy precursors to strengthen biological interpretation and develop more robust predictive models (Irawan *et al*., 2026).

Overall, milk yield divergence in Saanen dairy goats under similar feeding and management conditions was associated with distinct systemic metabolic phenotypes. HP goats were characterized by lower NEFA, BHBA, and cortisol concentrations and higher abundance of metabolites related to carbohydrate activation, one-carbon metabolism, and riboflavin-linked pathways. In contrast, LP goats showed higher lipid mobilization and ketogenesis indicators, glucocorticoid-related metabolites, kynurenine pathway metabolites, methylmalonic acid, and 1-methylhistidine. These findings indicate that serum metabolomics, when combined with conventional metabolic and hormonal indicators, may help characterize production-associated metabolic differences in lactating dairy goats and support the future development of metabolite-informed nutritional evaluation strategies (Islamiyati *et al*., 2025; Irawan *et al*., 2026).

CONCLUSION

Lactating Saanen dairy goats with higher milk production exhibited a more favorable metabolic phenotype characterized by improved energy balance, reduced metabolic stress, and enhanced carbohydrate and redox metabolism, whereas lower-producing goats showed metabolic signatures associated with lipid mobilization, ketogenesis, protein catabolism, and stress-related pathways. The identified metabolite biomarkers provide promising indicators for distinguishing metabolic efficiency between production groups. These findings support the application of metabolomic profiling as a practical tool for the early assessment of metabolic status and for developing targeted nutritional and herd management strategies to improve milk production, animal health, and the sustainability of dairy goat production systems.

CONFLICT OF INTEREST

The authors declare no conflicts of interest, financial or personal, that could have influenced the work reported in this manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the writing process, none of the authors used generative AI or AI-assisted technologies.

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