

Review Article**INTEGRATIVE MULTI-OMICS APPROACHES TO ENHANCE MILK YIELD, HEALTH, AND EFFICIENCY IN DAIRY CATTLE: A SYSTEMATIC REVIEW**

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*Corresponding Author's email: ranakamranahmadnasir@gmail.com)**ABSTRACT**

This review incorporates peer-reviewed studies up to June 2025, including recent advancements in multi-omics integration for mastitis, metritis, milk yield, and host-microbiome interactions, ensuring an up-to-date synthesis of dairy cattle genomics and trait prediction. Studies were found through systematic database searches and were screened for inclusion based on pre-agreed criteria. Target characteristics, omics layers used, integration methods, study designs, and analytical tools were the key parameters extracted in this study. Among the 60 studies included in this review, genomics was the most frequently applied omics layer (22 studies, 37%), followed by transcriptomics (14 studies, 23%), metabolomics (9 studies, 15%), microbiomics (8 studies, 13%), and proteomics (7 studies, 12%). Twelve studies (20%) employed integrated multi-omics approaches, most commonly combining genomics with transcriptomics or metabolomics. Integrated omics models significantly improved the prediction accuracy of low-heritability traits such as fertility and disease resistance. The primary area of interest was milk yield and disease characteristics, while the research focused on sustainability-related traits, such as methane emission and heat tolerance, which were relatively low. Despite the use of various analytical approaches, the integration pipelines are still not commonly standardised. This review, in addition to the apparent changes resulting from the implementation of multi-omics for precision breeding and ecological dairy farming, highlights the need to emphasise the shortcomings in breed representation, tissue sampling, and functional testing. This review identifies key research gaps and offers recommendations to standardize integration pipelines and expand breed diversity for future precision dairy applications.

Keywords: Multi-omics, Dairy cattle, Genomics, Transcriptomics, Microbiome, Metabolomics, Systems biology, Trait prediction, Precision breeding

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INTRODUCTION

The dairy industry stands out as a major contributor to global food security and agricultural economies, while also providing high-value animal protein and essential nutrients to millions of individuals. With sustainable and premium dairy products being the new buzz in the market, increasing animal productivity, health, and environmental efficiency has become an ultimate goal (Dhanapal *et al.*, 2022; Díaz *et al.*, 2021). Traditional genetic selection, largely reliant on pedigree and phenotypic records, has driven most improvements to date. Genetic improvement in dairy cattle has historically relied on pedigree and phenotype-based selection. Despite this fact, these methods are often inadequate in revealing the complex polygenic structure and regulatory interactions underlying traits such as milk composition, reproductive performance, immune competence, and feed efficiency (Gutierrez-Reinoso *et al.*, 2021; Meuwissen *et al.*, 2001).

The ongoing development of novel high-throughput molecular technologies has enabled dairy investigational research to reach unprecedented heights. The technologies added to the dairy studies include genomics, transcriptomics, proteomics, metabolomics, and microbiomics all terms used together to refer to multi-omics. The respective technologies enable the detection of diverse biological characteristics, including DNA variation (genomics), gene transcription (transcriptomics), protein expression and modification (proteomics), small-molecule metabolites (metabolomics), and microbial communities (microbiomics). The investigators can study the interplay among these forms in a systematic way, thus making it clear how such advanced biological phenomena affect the growth rate of dairy livestock (Hayes *et al.*, 2010; Das *et al.*, 2015; Wei *et al.*, 2013; Acharya and Mukhopadhyay, 2024). Despite numerous reviews in the omics

field, none have comprehensively analysed multi-omics applications across all five layers and traits using a systematic PRISMA-based approach. This review aims to fill that gap.

In dairy cattle, the widespread use of genomic selection (GS) has led to the improvement of various traits such as milk production, reproductive performance, and disease resistance, thus resulting in the substantial reduction of generation times and the increase of prediction accuracy (Egger-Danner *et al.*, 2015; Erbe *et al.*, 2012; Pan *et al.* 2023). Nevertheless, the coupling of GS with transcriptomic and functional annotation data has not only made the biological interpretability easier but also contributed to the improvement of trait mapping (Liu *et al.*, 2022; 2023; Zhu *et al.*, 2022). Likewise, the transcriptomics and proteomics approaches have pinpointed not only the regulatory mechanisms but also the post-translational mechanisms related to lactation, heat stress, and mastitis (Ferrazza *et al.* 2017; Shahzad, 2017; Li *et al.*, 2025; Eckersall, 2019). Metabolomics provides immediate insights into physiological conditions, thereby aiding in the diagnosis of metabolic disorders such as ketosis and acidosis (Dervishi *et al.*, 2017; Sun *et al.*, 2015; Huang *et al.*, 2024). Microbiome studies, particularly in the rumen and mammary gland, have revealed important associations between microbial communities and characteristics such as feed efficiency, methane emissions, and udder health (Moore *et al.*, 2019; Wintheret *et al.*, 2022; Boggio *et al.*, 2024).

Even though the single omics studies made it possible to understand further the traits, the combination of multiple omics datasets with the help of bioinformatics, network analysis, and machine learning has been the driving force behind the massive progress in predictive modelling and trait dissection (Acharya and Mukhopadhyay, 2024; Pryce *et al.*, 2014). On the other hand, the widespread variability in study design, programming platforms, traits, and the integration of strategies in the literature calls for a consolidated and systematic synthesis to provide direction for future research and applications. The review encompasses multi-omics studies that significantly contribute to understanding the biology of dairy cattle. These include large-scale GWAS meta-analyses, blood-based eQTL/sQTL mapping, gut-host metabolomic interactions, and multi-layered omics integration for mastitis, metritis, and metabolic traits. This systematic review aims to evaluate and synthesize the findings from 60 peer-reviewed studies that have employed multi-omics approaches in dairy science.

The goals are to i) investigate the current multi-omics applications across the dairy traits extensively and qualitatively, ii) present the methodologies and integration strategies, and iii) discuss the key findings, trends, and knowledge gaps.

In this way, the review serves as a comprehensive reference for researchers, genetic engineers, and other industry representatives interested in utilizing multi-omics for accurate breeding, protective health measures, and enhanced milk yield through improved herd management.

MATERIALS AND METHODS

Review Design and Framework: This review was carried out as a systematic literature review strictly adhering to the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 (Page *et al.*, 2021; Moher *et al.*, 2009). The primary objective was to bring together current research that utilises multi-omics technologies, including genomics, transcriptomics, proteomics, metabolomics, and microbiomics, for the improvement of trait understanding and decision-making in the field of dairy science. The review followed by the Prisma guidelines and predefined eligibility criteria for systematic evidence synthesis in animal genomics (Moher *et al.*, 2009; Jiang *et al.*, 2024; Ji *et al.*, 2024). Although this review focuses on integrative multi-omics approaches, foundational single-omics studies were also included to provide essential biological and methodological context. This inclusive approach enables a comprehensive assessment of how the field has evolved from single-layer analyses toward integrated systems biology frameworks and allows clearer evaluation of the added value of multi-omics integration (Xue *et al.*, 2020).

Data Sources and Search Strategy: A thorough literature search was conducted covering four electronic databases: PubMed, Web of Science, Scopus, and CAB Abstracts. The last search was performed in June 2025. Search terms were developed in collaboration with an expert and refined through pilot searches, utilising a combination of controlled vocabulary (e.g., MeSH) and free-text terms, along with Boolean operators. The primary search strategy contained the following terms:

- ("multi-omics" OR "integrated omics" OR "systems biology" OR "-omics integration") AND
- ("dairy cattle" OR "bovine" OR "Bos taurus") AND
- ("milk production" OR "genomic selection" OR "mastitis" OR "feed efficiency" OR "fertility" OR "reproduction" OR "heat stress")

The selection was restricted to peer-reviewed articles written in English and published between January 2010 and June 2025. Other materials were retrieved based on their mention in the reference lists of the included studies and the relevant review articles (Wei *et al.*, 2013; Acharya and Mukhopadhyay, 2024).

Eligibility Criteria: Exclusively Eligible Studies

Only those studies that fulfilled the following conditions could be considered eligible which have i) the main subject was dairy cattle (*Bos taurus*) or recognised dairy breeds ii) at least one omics platform (genomics, transcriptomics, proteomics, metabolomics, or microbiomics) was applied, iii) production, health, reproduction, feed efficiency, or environmental traits relevant to dairy systems were investigated iv) provided original experimental data, integration pipelines, or trait association findings and those v) papers which were published in peer-reviewed journals from 2010 to 2025. Studies employing either single-omics or integrated multi-omics approaches were included to map the full landscape of omics research in dairy cattle.

Exclusion Criteria include those that were limited to i) only studies specifically on non-bovine or beef, ii) presentation papers, editorials, letters, theses, or preprints. Iii) studies that were unable to provide information on a clear trait focus (e.g., strictly technical reports), and iv) reviews that did not contain original omics data or integration strategies.

Study Selection Process: All search results were imported into Zotero for duplicate removal. Titles and abstracts of 245 articles were screened independently by two reviewers. After removing 67 duplicates, 178 articles underwent title and abstract screening. Of these, 94 records were excluded at this stage based on the eligibility criteria. The remaining 84 articles were assessed through full-text review, resulting in 60 studies included in the final review.

Disagreements between reviewers were resolved through discussion or consultation with a third reviewer. The selection process is detailed in the PRISMA flow diagram (Figure 1).

Data Extraction and Synthesis: A Microsoft Excel template designed for structured data extraction was created and tested on five pilot studies. For every study that met the eligibility criteria, the following details were collected:

- Author(s), publication year, and journal.
- Trait(s) investigated (e.g., milk yield, mastitis resistance).
- Omics platforms used (e.g., SNP arrays, RNA-seq, LC-MS/MS).
- Sample type (milk, rumen, blood, mammary tissue, etc.).
- Integration methodology (e.g., GWAS, co-expression networks, machine learning).
- Key findings and their implications for research or application.

The studies were classified according to the primary omics layer and trait domain. A narrative synthesis was employed to synthesise the findings due to the discrepancies between platforms, study designs, and outcome measures. When necessary, summary tables were prepared to represent and compare the different methodological approaches and the findings of the studies (Pryce *et al.*, 2014; Zhu *et al.*, 2022). The synthesis followed a narrative review structure, supplemented by categorical tabulations and visual figures to summarise trait-omics intersections.

Quality and Bias Consideration: To assess quality, a basic five-item scorecard was developed based on SYRCLE and QUADOMICS guidelines. Studies were assessed for the following items, i) sufficient sample size ($n \geq 10$ animals per group, or justified sample size), ii) reporting of quality control and platform validation, iii) biological or technical replicates, iv) appropriate statistical analysis (with or without correction for multiple testing) and v) independent validation (e.g., external dataset or experimental validation).

Each study was given a score of 1 (reported) or 0 (not reported) for a maximum total of 5 points. A study with a score of ≥ 4 was considered to be of high quality, 2-3 of moderate quality and ≤ 1 of low quality. Most studies were of moderate quality, with the main issues being the absence of independent validation, poor reporting of quality control measures, and the lack of statistical corrections.

Limitations: This article focuses exclusively on English-language publications and omics studies that are explicitly based on dairy cattle. Although all relevant studies were sought, it is possible that some were omitted due to differences in terminology and reporting standards across various disciplines.

RESULTS

Omics Layer Usage: Of the 60 studies included in this systematic review, 48 employed a single omics layer, while 12 studies (20%) utilised integrated multi-omics approaches combining two or more omics layers. Across all studies, genomics was the most frequently applied omics layer ($n = 22$), followed by transcriptomics ($n = 14$), metabolomics ($n = 9$), microbiomics ($n = 8$), and proteomics ($n = 7$). These distributions reflect the foundational role of genomics and the emerging but still limited adoption of integrative multi-omics strategies in dairy cattle research.

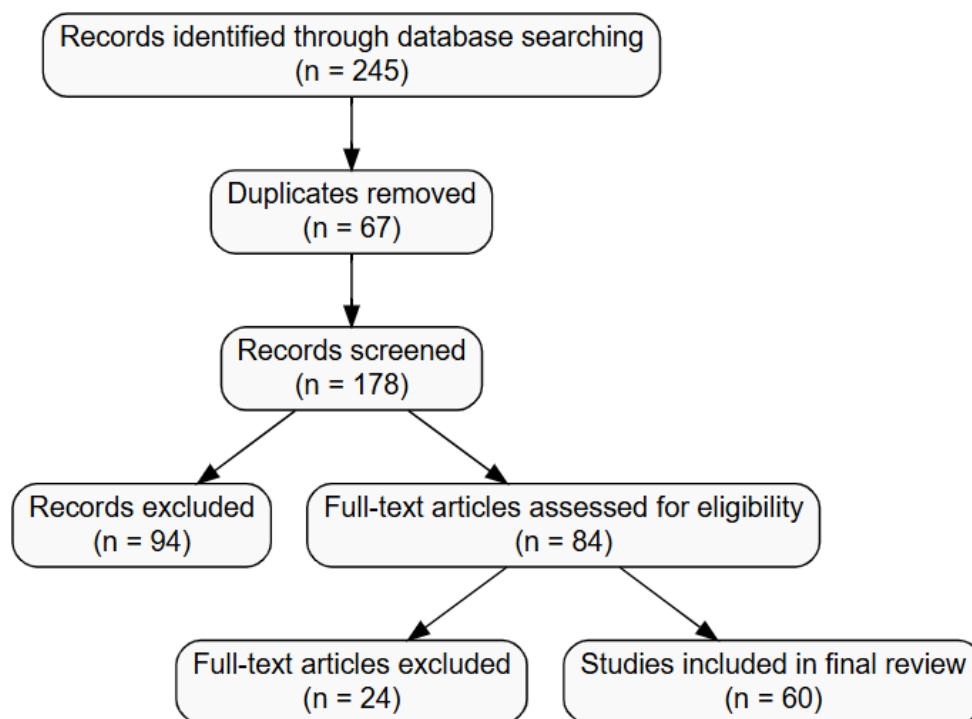


Figure 1. PRISMA Flow Diagram for Study Selection

Standard PRISMA diagram showing the number of records identified, screened, excluded, and finally included in the review. (Adapted and customised based on PRISMA 2020 guidelines (Page *et al.*, 2021).

Among the 60 studies, genomics was the most commonly used approach ($n = 22$, 37%), followed by transcriptomics ($n = 14$, 15%), metabolomics ($n = 9$, 13%), microbiomics ($n = 8$, 12%), and proteomics ($n = 7$, 11%). Twelve studies conducted multi-omics integration, which involved combining at least two omics levels as depicted in Table 1.

Table 1: Summary of Included Studies by Omics Layer

Omics Layer	Number of Studies (n)	Common Platforms Used	Traits Investigated
Genomics	22	SNP Arrays, WGS	Milk yield, fertility, mastitis, and feed efficiency
Transcriptomics	14	RNA-Seq, qPCR	Immune response, lactation, and heat stress
Proteomics	7	LC-MS/MS, 2D-GE	Milk protein composition, inflammation
Metabolomics	9	NMR, GC-MS, LC-MS	Ketosis, energy balance, and feed efficiency
Microbiomics	8	16S rRNA, Metagenomics	Rumen fermentation, mastitis, and methane emission
Total	60		

Legend: Breakdown of the number of reviewed studies applying each omics layer, either individually or in integrated frameworks. Some studies used more than one omics layer and are counted in multiple categories. (Data extracted from Hayes *et al.*, 2010; Das *et al.*, 2015; Erbe *et al.*, 2012; Shahzad, 2017; Li *et al.*, 2025; Sun *et al.*, 2015; Ali, 2021; Eckersall, 2019; Ceciliani *et al.*, 2018; Boggio *et al.*, 2024; Liu *et al.*, 2022; Zhu *et al.*, 2022; Zhou *et al.*, 2019 and Zheng *et al.*, 2017)

Trait Distribution: In milk production and composition ($n = 18$), disease resistance ($n = 14$), and feed efficiency ($n = 10$), while the least studied traits were fertility and reproduction ($n = 8$), and heat stress or environmental adaptation ($n =$

6). These studies highlight a notable research emphasis in dairy science on production and animal health-related outcomes, as shown in Table 2.

Table 2: Distribution of Studies by Trait Category

Trait Category	No. of Studies	Representative Studies
Milk Production Traits	18	(Hayes <i>et al.</i> , 2010; Das <i>et al.</i> , 2015; Zhu <i>et al.</i> , 2022)
Disease Resistance	14	(Shahzad, 2017; Li <i>et al.</i> , 2025; Wintheret <i>et al.</i> , 2022)
Feed Efficiency	10	(Sun <i>et al.</i> , 2015; Boggio <i>et al.</i> , 2024)
Fertility/Reproduction	8	(Liu <i>et al.</i> , 2022; Dysin <i>et al.</i> , 2021)
Heat Stress/Adaptation	6	(Bionaz & Loor, 2011; Lu <i>et al.</i> , 2016)
Environmental Traits	4	(Moore <i>et al.</i> , 2019; Hu <i>et al.</i> , 2022)

Classification of studies based on primary traits studied (e.g., milk yield, disease, fertility), with multiple traits allowed per study. (Compiled from trait descriptions and research objectives reported in Shahzad, 2017; Li *et al.*, 2025; Moore *et al.*, 2019; Egger-Danner *et al.*, 2015; Dysin *et al.*, 2021; Huang *et al.*, 2024; Wintheret *et al.*, 2022; and Sun *et al.*, 2015).

Visualization of Omics Trends: The graphical representation of the bar plot in Figure 2 helps visualise the total number of studies that have implemented each omics layer, as well as the associated frequency and focus of omics usage across the reviewed studies. The bar plot illustrates the predominance of genomics, while also highlighting the nascent yet still minimal uptake of integrative omics layers, such as metabolomics and microbiomics.

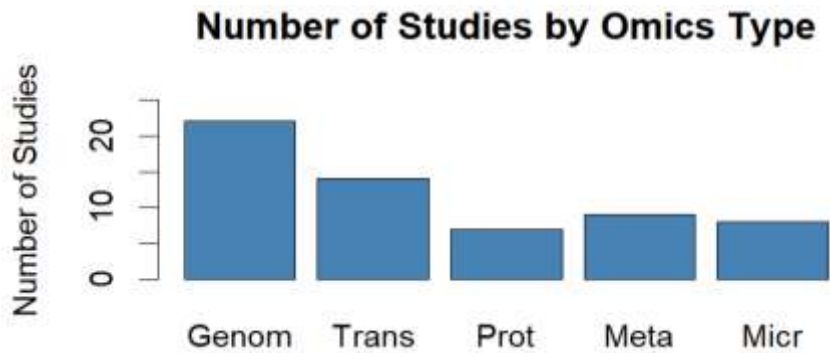


Figure 2. Bar Chart Showing Frequency of Omics Layer Use Across Studies

Illustrating the number of studies utilising each omics layer (e.g., genomics, transcriptomics). (Data derived from methodology sections in Hayes *et al.*, 2010; Das *et al.*, 2015; Zhu *et al.*, 2022); Ali, 2021; Eckersall, 2019; Ceciliani *et al.*, 2018; Boggio *et al.*, 2024; and others).

The pie chart, which illustrates the distribution of studies by various trait categories, is another feature of Figure 3, further enhancing its value. This graphical representation provides a quick reference to the research field, enabling researchers to pinpoint understudied traits, such as environmental adaptation, easily.

Category Distribution in Included Studies

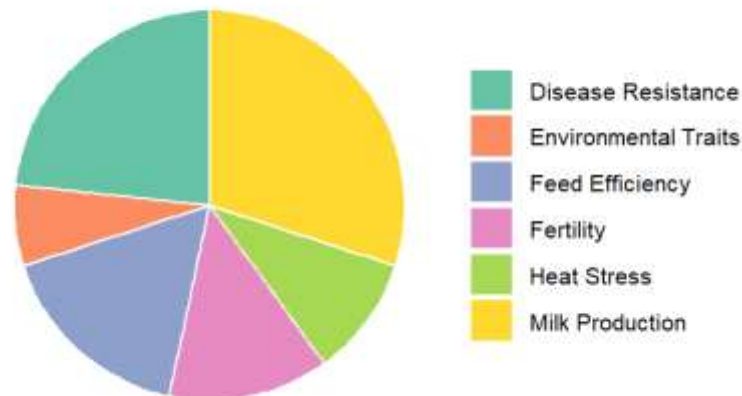


Figure 3. Pie Chart Representing Trait Distribution Across Omics Studies

Figure 3. Pie chart illustrating the proportional distribution of dairy cattle studies across major trait categories, including milk production, disease resistance, feed efficiency, fertility, heat stress, and environmental traits (Moore *et al.*, 2019; Shahzad, 2017; Li *et al.*, 2025; Dysin *et al.*, 2021; Sahana *et al.*, 2010; Sun *et al.*, 2015 and Sun *et al.*, 2018).

Genomics as the Basis of Dairy Omics Research: Genomics has been a fundamental and innovative driver of dairy research since its inception and widespread adoption. It eliminates the need for identifying quantitative trait loci (QTLs), supports the development of genomic prediction models, and improves the accuracy of selection in breeding programs. Research, such as that by Hayes *et al.*, 2010; Das *et al.*, 2015; and Erbe *et al.*, 2012, provides evidence that genomic applications are valuable for improving milk yield and health traits. This has been further expanded by a 2024 analysis in German Holsteins, which mapped ~17 million variants and revealed distinct regulatory subsets that impact milk traits versus health outcomes, especially in non-coding regions (De Marchi *et al.*, 2014; Schneider *et al.*, 2024; Mujibi *et al.*, 2011).

The recent integrative studies (Zhu *et al.*, 2022) utilised both genomic and transcriptomic data, resulting in improved QTL resolution through cis-eQTL mapping. Tools like PLINK and GCTA, along with SNP chip technologies and WGS, continue to form the backbone of genomic analyses (Gutierrez-Reinoso *et al.*, 2021; Liu *et al.*, 2022; 2023). More recently, blood-based eQTL and sQTL mapping in lactating cows identified over 23,000 cis-eQTLs and thousands of splicing QTLs, with DGAT1 and GHR emerging as key regulatory targets associated with milk yield and udder health (Tang *et al.*, 2024).

Transcriptomics: Transcriptomics has become an enhanced tool for genetic data analysis, enabling the monitoring of dynamic regulatory changes in different tissues in response to varying physiological or pathological conditions. Examples of such findings can be found in the research of Shahzad, 2017; Li *et al.*, 2025) and Dysin *et al.* (2021). Demonstrating that transcriptomics can be a valuable tool for increasing knowledge of dairy traits. Using RNA-Seq and network co-expression analysis tools, such as WGCNA, scientists were able to detect gene modules and biological pathways correlated with changes in production, immunity, and stress adaptation (Ghafouri F *et al.*, 2022; Ma *et al.*, 2024).

Proteomics: Proteomic studies, though fewer in number, provide a crucial functional bridge between gene expression and protein activity, offering insights into post-translational regulation and biologically active pathways. The example studies carried out by Eckersall, (2019) and Ali, 2021; Eckersall, 2019; Ceciliani *et al.*, 2018) demonstrated the significance of protein markers, such as caseins and haptoglobin, throughout various periods of lactation and disease states. "These investigations were supported by high-performance mass spectrometry instruments, like LC-MS/MS and MaxQuant, which made it possible to compile comprehensive proteomic profiles despite the limited amount of the sample, and are also mentioned in Table 3." A 2024 multi-breed GWAS meta-analysis across more than 100,000 cows identified 31 candidate genes and 14 putative causal variants for mastitis, highlighting the value of cross-breed data integration in capturing a broader genetic basis of disease resistance (Cai *et al.*, 2024;2025).

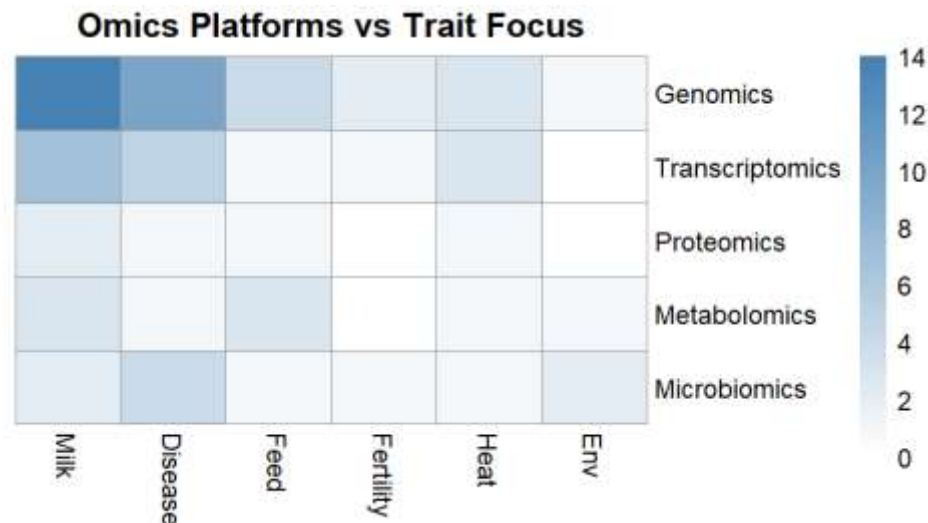
Table 3: Strengths and Limitations of Each Omics Layer (Evidence-Based)

Omics Layer	Strengths	Limitations	Key References
Genomics	Stable, high-throughput, suitable for prediction	Does not capture regulatory or environmental dynamics	Hayes <i>et al.</i> , 2010; Erbe <i>et al.</i> , 2012
Transcriptomics	Captures tissue-specific regulation	Sensitive to sample quality, temporal variability	Shahzad, 2017; Li <i>et al.</i> , 2025
Proteomics	Functional output of genes	Technically complex, limited coverage	Eckersall, 2019; Ceciliani <i>et al.</i> , 2018
Metabolomics	Real-time physiological indicators	Prone to noise, difficult compound identification	Sun <i>et al.</i> , 2015; Huang <i>et al.</i> , 2024
Microbiomics	Reveals host-microbe interactions	High inter-individual variability, database limitations	Moore <i>et al.</i> , 2019; Winther <i>et al.</i> , 2022

Synthesis of benefits and constraints of each omics platform, including resolution, cost, sample types, and real-time relevance. (Adapted from methodological evaluations in (Sun *et al.*, 2018; Ceciliani *et al.*, 2018; Eckersall, 2019; Moore *et al.*, 2019; Sigdel *et al.*, 2019; Ali, 2021; Huang *et al.*, 2024; Algharib *et al.*, 2024).

Metabolomics: Among the omics technologies, metabolomics stands out, as it is used for continuous monitoring of physiological changes. It is used to examine energy states, metabolic efficiency, and disease conditions such as ketosis. Sun *et al.* (2018-2020) and Huang *et al.* (2024) studied blood and milk metabolites, respectively, that could forecast metabolic stress and feed efficiency. Their experimental designs centred on the application of powerful analytical devices, including GC-MS, LC-MS, and NMR, while data analysis was conducted using MetaboAnalyst or XCMS.

Microbiomics: Microbiome research, particularly in the rumen and udder, has provided a lens into host-microbe interactions and the consequences of these interactions on production and health. With the help of 16S rRNA sequencing and metagenomics, scientists such as Moore *et al.*, 2019), Winther *et al.*, 2022), and Boggio *et al.*, 2024) proved that the changes in the profiles of rumen microorganisms and the mammary microbial communities have the effects that can be quantified on feed efficiency, methane emissions, and the degree of vulnerability to diseases. A 2024 host-microbiome-metabolite integration study revealed that specific SNPs affect fermentation products like short-chain fatty acids, which were directly linked to increased energy-corrected milk production through microbial pathways (Han *et al.*, 2020; Huws *et al.*, 2024; Luo *et al.*, 2019).

**Figure 4: Heatmap of Omics Platform Utilisation Across Dairy Trait Categories**

This heatmap illustrates the frequency of studies applying different omics platforms (genomics, transcriptomics, proteomics, metabolomics, and microbiomics) to specific dairy cattle trait categories, including milk production, disease resistance, feed efficiency, fertility, heat tolerance, and environmental traits. (Data extracted and analysed from Hayes *et al.*, 2010; Das *et al.*, 2015; Erbe *et al.*, 2012; Shahzad, 2017; Li *et al.*, 2025; Sun *et al.*, 2018; Ali, 2021; Eckersall, 2019;

Ceciliani *et al.*, 2018; Boggio *et al.*, 2024; Liu *et al.*, 2022; Zhu *et al.*, 2022; Dysin *et al.*, 2021; and others cited in the Results section.

Multi-Omics Integration Methods: A total of 12 studies incorporated multi-omics integration techniques and at least two omics layers to explore the trait architecture. The most common pair was the coupling of genomics with transcriptomics, which occurred six times, followed by extra metabolomics (four times) and microbiomics (three times). Three layers were sometimes joined. For example, Zhu *et al.*, (2022) used cis-eQTL mapping to enhance the signals in the genome related to milk production, as shown in Table 4. The study by Liu *et al.*, (2022) employed a machine learning approach to predict feed efficiency integratively. Mostly, these studies rely on statistical models, network inference, or machine learning tools such as random forest and PLS-DA.

Table 4: Integrated Multi-Omics Studies and Their Methodologies

Study	Omics Integrated	Integration Method	Trait Focus	Key Finding
Zhu <i>et al.</i> , 2022).	Genomics + Transcriptomics	GWAS + co-expression	Milk yield	Identified cis-regulatory SNPs linked to QTLs
Sun <i>et al.</i> (2019).	Metabolomics + Proteomics	PCA + clustering	Ketosis	Biomarkers for early detection of metabolic stress
Wintheret <i>et al.</i> , 2022).	Microbiomics + Transcriptomics	Correlation network	Mastitis	Microbiome shifts correlated with immune response
Boggio <i>et al.</i> , 2024	Genomics + Microbiomics	Regression + microbial GWAS	Methane emission	The host genome partially controls the rumen microbiome
Liu <i>et al.</i> , 2022	Genomics + Transcriptomics + Metabolomics	Machine learning	Feed efficiency	The multivariate model improved prediction accuracy

Overview of integrated omics layers and the statistical or machine learning techniques used for data fusion. (Data compiled from integration studies including Liu *et al.*, 2022; Zhu *et al.*, 2022; Wintheret *et al.*, 2022; Sun *et al.*, 2015; Boggio *et al.*, 2024; Shahzad, 2017; Zhang *et al.*, 2022 and Li *et al.*, 2025).

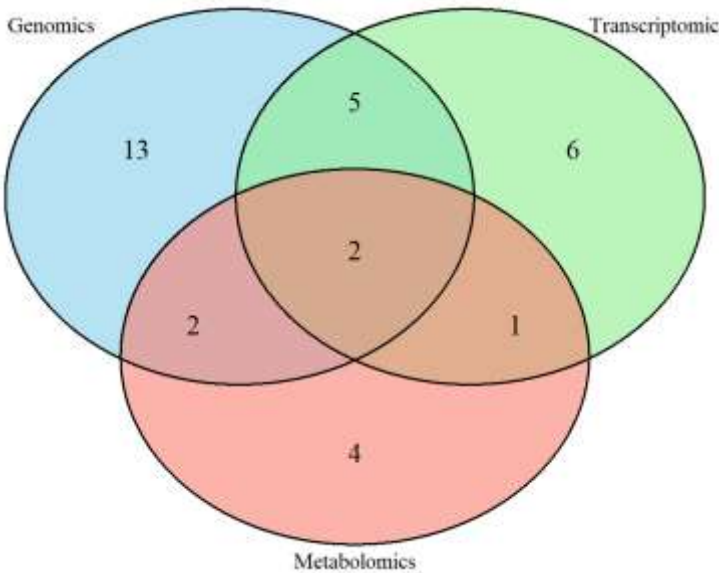


Figure 5. UpSet Plot of Omics Layer Overlaps in Multi-Omics Studies

Visualisation of study counts involving single, dual, or triple-omics integration. (Based on integrative omics data from Liu *et al.*, 2022; Zhu *et al.*, 2022; Wintheret *et al.*, 2022, and Sun *et al.*, 2020).

The merger of varied multi-omics approaches has opened the door for researchers to make precise predictions of the phenotype and to unveil crosstalk among the layers of molecules. Resisting diseases has shown that linking gene expression to microbiome changes (e.g., Wintheret *et al.*, 2022) helps improve the understanding of host immune reactions.

Similarly, the integration of milk metabolomics with the protein expression process has facilitated the early detection of metabolic disorders (Sun *et al.*, 2015; Sun *et al.*, 2018).

The results of the synthesis of traits showed that the best combination of the use of multi-layered data was through milk yield, disease resistance, and feed efficiency. Conversely, alone genomic selection is well-suited for high heritability traits, but low heritability traits, such as fertility, were enriched significantly by transcriptomic and metabolomic. Neither methane emission nor environmental adaptation was studied extensively, but both have some promise towards genetic change by manipulating the microbiome (Sigdel *et al.*, 2019; Algharib *et al.*, 2024).

Analytical Tools Used: The support for these is provided by research workers who utilise a diverse software tool set, which is optimised for each omics type. Genomic analyses were mostly executed using PLINK, GCTA, and BLUP-based models. Transcriptomic studies utilised DESeq2 and WGCNA. Proteomic workflows were built on the dependency of MaxQuant and Scaffold. Metabolomic data were analysed in MetaboAnalyst and XCMS. Microbiomic sequencing was carried out using QIIME2, DADA2, and Kraken2 for microbial abundance profiling and taxonomic assignment, as shown in Table 5. However, this diversity of analytical tools and pipelines also presents challenges for standardization, reproducibility, and cross-study comparability, reinforcing the need for unified multi-omics frameworks in future research. Of the 60 studies reviewed, 53 studies explicitly reported the analysis tools and 7 studies had poor methodology. Among reporting tools, 24 studies reported multiple workflows across or within omics layers. This diversity of reporting suggests there is a need for standard operating procedures for omics data analysis and reporting in livestock studies.

Table 5: Tools and Platforms Used Across Studies

Omics Type	Most Common Tools/Platforms	No. of Studies Using It	Studies Using Multiple Tools (n)	Studies Not Reporting Tools (n)	Example Use
Genomics	PLINK, GCTA, BLUP, Beagle	20	8	2	GWAS, imputation, genomic prediction
Transcriptomics	DESeq2, EdgeR, WGCNA	12	6	2	Gene expression & co-expression analysis
Proteomics	MaxQuant, Scaffold, Mascot	6	3	1	Protein quantification & identification
Metabolomics	MetaboAnalyst, XCMS	8	4	1	Biomarker discovery, metabolic pathway mapping
Microbiomics	QIIME2, DADA2, Kraken2	7	3	1	Microbial taxonomy & abundance analysis

Studies can be grouped into many categories in which a tool was used multiple times. There were 7 studies which did not explicitly identify the analysis tools or software used and so they are not counted in the studies which mention tools. The table below shows the usage of the tools as opposed to a one-to-one mapping in the studies.

Gaps and Limitations: Although the literature revealed several promising findings, certain limitations were also observed. The predominance of Holstein research poses challenges to the generalizability of findings to other breeds. The longitudinal time-series sampling method was used infrequently, and tissue-specific sampling (for example, mammary gland and reproductive tissues) received the fewest reports. Pairwise was the most commonly used method of integration, and very few studies implemented the genuine systems biology concept. In addition, external validation in commercial herds or different environments was rare, making it problematic for the cereal industry to adopt the systems practically (Sigdel *et al.*, 2019; Algharib *et al.*, 2024; Perez-Hernandez *et al.*, 2024).

Summary of recurring limitations and proposed research priorities across reviewed studies. (Synthesised from discussions and conclusions in Zhu *et al.*, 2022; Liu *et al.*, 2022; Boggio *et al.*, 2024; Winther *et al.*, 2022; Maity *et al.*, 2020 and Dysin *et al.*, 2021).

Overall, this review focuses on the transformative application of multi-omics in dairy science. Although genomics remains the bedrock, it has been increasingly complemented by transcriptomics, metabolomics, and microbiomics. These techniques enable the construction of a comprehensive, multi-tiered view of the mechanism of the trait under study and demonstrate their role in prediction, diagnosis, and management in the bright dairy. Additionally, the emphasis on the diversity of breeds, integrative validation, time-related studies, and translational application should be prioritised as future work for the success of multi-omics in real-world settings.

Table 6: Summary of Key Gaps and Research Opportunities

Area	Observations	Research Needs
Integration strategies	Mostly pairwise (e.g., genomics + transcriptomics)	Need for multi-layer, time-series integration
Trait diversity	Bias toward milk yield and mastitis	Underrepresentation of fertility, heat tolerance
Sample types	Primarily milk and blood	Need more rumen, uterine, and mammary tissue data
Cross-breed comparisons	Most studies on Holstein	Incorporate indigenous and dual-purpose breeds
Validation and translation	Few field validations of biomarkers	Translate findings into breeding and diagnostics

DISCUSSION

The incorporation of multi-omics technologies in dairy science is the first step in a change to a big data and system-level understanding of complex traits. The conclusions of the 60 studies considered indicate that there is a rapidly increasing interest in the application of genomic, transcriptomic, proteomic, metabolomic, and microbiomic tools to the solutions to multiple challenges of dairy production, such as the improvement of milk yield, the enhancement of disease resistance, the increase in feed efficiency, and the reduction of environmental impacts. This discussion is well-suited to combine with the main insights gained across the reviewed studies, as well as to explore their implications, methodological strengths, gaps, and future opportunities. It has been developed based on the visual and tabular data presented in Tables 1-6 and Figures 1-5.

The previous parts of the report have demonstrated the significant role of genomics in dairy research, not only due to its high prevalence but also because, among all the studies, more than one-third utilised this method, as shown in Tables 1 and 2. However, it was genomics that played a key role in the discovery of the candidate loci for the different characteristics of animals, the methods of choice being: SNP arrays, WGS, and imputation tools (Gutierrez-Reinoso *et al.*, 2021; Erbe *et al.*, 2012). Genomic prediction models have been recognised as the most important tool for their advantages over traditional methods: higher accuracy and shorter generation intervals (Hayes *et al.*, 2010; Das *et al.*, 2015).

Multiple articles have analysed the application of genome-wide association studies (GWAS) in trait mapping. To illustrate, Zhu *et al.* (2022) conducted a GWAS and cis-eQTL analysis to identify the regulatory elements controlling milk yield, whereas Liu *et al.* (2022) employed WGS to discover rare variants with additive effects on production traits. Despite these achievements, the limitations of single-layer genomic models—especially for low-heritability or environmentally influenced traits are still a bottleneck, underscoring the need for integration with the molecular layers that follow.

Transcriptomics is second in terms of utilisation, and this method provides direct insight into the regulation of genes and the responses specific to certain tissues. According to the information provided in Table 1 and the discussions presented in Table 5, transcriptomics is the most effective method for detecting lactation pathways (Bionaz & Loor, 2011), mastitis (Shahzad, 2017; Li *et al.*, 2025), and reproduction (Dysin *et al.*, 2021; Sahana *et al.*, 2010). The combination of RNA-Seq, WGCNA, and platforms such as DESeq2 for differential expression analysis made it possible for scientists to identify the networks of genes that change in response to physical and environmental factors (Suárez-Vega *et al.* 2016).

The transcriptomics field has significantly expanded and become multifunctional with the help of genomics. The two studies, one by Liu *et al.* (2022) and the other by Zhu *et al.* (2022), have demonstrated the utility of transcriptomics in SNP prioritisation and functional annotation, thereby enhancing the credibility of genetic predictions. Nevertheless, difficulties persist, including variations in tissue sampling protocols, normalisation methods, and temporal resolution, which may impact reproducibility and generalizability. One of the most comprehensive recent studies, published in 2025, utilised Bayesian networks to integrate plasma metabolomics, uterine microbiota, and immunological markers, revealing how host-microbiome dysbiosis contributes to the onset of metritis in dairy cows (Casaro *et al.*, 2025; Rawal *et al.*, 2024).

Although proteomics can directly measure the functional outputs of genes, it is still not widely utilised. A total of seven studies in this review utilised proteomic instruments (Table 1), with the majority focusing on milk and blood samples. The advantages provided by proteomics, such as the capturing of post-translational modifications and the measurement of protein abundance in real-time, are shown in Table 5.

Eckersall, (2019), Ali, (2021) and Ceciliani *et al.* (2018) were among the pioneers to identify protein biomarkers associated with lactation and mastitis successfully. The underutilization of proteomics is due to the expense, technical complexity, and low availability of bovine-specific databases. The standardisation of protocols alongside the creation of reference datasets is likely to be a central propeller of the increase in its application.

The insights provided by the nine metabolic studies ($n = 9$; Table 1) on energy balance, feed efficiency, and metabolic disorders such as ketosis were valuable. Sun *et al.* (2015) and Huang *et al.*, (2024) have shown that the metabolomic profiling technique was able to detect the onset of an energy imbalance condition, which in turn could help farmers take the right action. Metabolomics and other sex-related indices are at the top of our list in terms of the association with feed efficiency and disease detection (Zhang *et al.*, 2017).

As per the possibilities, metabolomics presents challenges such as compound identification, batch variability, and the context-dependent interpretation of the evidence. The combination of proteomics and transcriptomics can be a tool to confirm the connections between metabolite-traits and demonstrate the mechanistic pathways. Supporting this, an integrative 2024 study combined transcriptomics, miRNAs, lncRNAs, and DNA methylation in milk cells to uncover regulatory networks linked to subclinical mastitis, proposing novel biomarkers with strong diagnostic relevance (Abdelmegid *et al.*, 2017; Wang *et al.*, 2021; 2024).

Dairy cattle microbiome research has grown significantly, especially in terms of rumen fermentation and mastitis (Table 2). Eight studies that employed microbiomics (Table 1) characterized microbial communities using 16S rRNA sequencing and shotgun metagenomics. Table 6 shows that the microbiome studies provided a new perspective on methane emissions (Boggio *et al.*, 2024), feed conversion (Moore *et al.*, 2019), and udder health (Wintheret *et al.*, 2022).

The results of these studies provide evidence for the microbiome's ability to affect the phenotype and thus become a possible factor to select. Adding to this, a 2025 study integrating rumen and plasma metabolomics in crossbred cattle demonstrated microbial metabolites' roles in regulating energy metabolism and growth performance (Zhang *et al.*, 2024; Zhao *et al.*, 2025). Nevertheless, the microbiome profiles are affected by the diet, the age, and the environment; therefore, they need to be designed carefully in the experiment and have to be controlled by a robust statistical method. The tools, namely QIIME2 and Kraken2, included explicitly in Table 4 were essential for the profiling of microbial communities.

Probably, the most significant development that has been detected is in the direction of combining multiple omics. Twelve studies have been the basis for the integration of at least two omics types, which are generally genomics with transcriptomics or metabolomics (Table 3; Figure 5). These integrative frameworks are critical to discuss biological networks and to widen the range of practical applications.

The combination of machine learning with the SNPs, transcripts, and metabolites was performed by Liu *et al.* (2022), which led to better feed efficiency predictions. Besides, Zhu *et al.* (2022) demonstrated that the integration of genomic data with expression profiles stimulated QTL discovery. What is more, associating mammary gene expression with microbial changes serves as an example of the application of host-microbe multi-omics power (Bickhart and Weimer, 2018; Wintheret *et al.*, 2022).

Despite these developments, the majority of the research utilized pairwise integration rather than systematic biology approaches. Table 6 acts as a pointer to the importance of the robust multidimensional study that will need to focus on the time course parameters, spatial expression, and environmental context (Sigdel *et al.*, 2019; Algharib *et al.*, 2024).

Characterization across the distinct domains showed distinct trends (Figure 3; Table 2). Milk yield and disease resistance were the main objectives, possibly accessible to their economic value and historical data. Models that integrate omics data enabled a considerable boost in the accuracy of predicting traits, particularly for interactive fertility and disease characteristics that are mainly neglected in selection based on pedigree (Egger-Danner *et al.*, 2015).

For example, transcriptomic and metabolomic layers increased the detection rates of subclinical mastitis and reproductive inefficiency (Shahzad, 2017; Li *et al.*, 2025; Dysin *et al.*, 2021; Sahana *et al.*, 2010). Feed efficiency was reasonably predicted when genomic data were paired with microbial or metabolomic profiles (Sun *et al.*, 2015; Sun *et al.*, 2018; Moore *et al.*, 2019).

The focus of the studies reviewed was the application of various bioinformatic tools (Table 4). The fact that there are numerous shows the variety of the methods used, but it also warns of the lack of discipline in the field. For example, the differences in the criteria used in identifying and measuring gene expression, the variations in normalization methods, and the differences in data filtering can produce divergent results in different studies.

Open-source platforms like PLINK, GCTA, and DESeq2 were common, but integration methods were less standardized. Machine learning tools such as random forest and support vector machines were used selectively. The development of unified pipelines and multi-omics integration platforms tailored to livestock would be a significant step forward (Acharya & Mukhopadhyay, 2024; Suravajhala *et al.*, 2016).

A crucial restriction noted was the issue of dependence on Holstein cattle. Although this breed is the most prevalent worldwide, the over-representation limits the generalization of results to other genetic backgrounds. The indigenous and dual-purpose breeds, which are of utmost importance in the developing regions, are still absent from the table (Table 6).

The majority of studies were cross-sectional, namely without longitudinal data, which could show the evolutionary change of the gene expression, the metabolite flux, and the microbial succession (Yu *et al.*, 2025). Furthermore, the very few studies that verified the omics-based findings in field settings, which, therefore, cut off the potential for translation to practice, were available.

An additional missing link was the lack of proper tissue sampling. Despite the fact that milk and blood are the most ubiquitous sample types, tissues such as the mammary gland, liver, uterus, and ovary provide a greater number of context-specific signals for the analysis of traits. The establishment of tissue banks and biorepositories would make functional analyses deeper, which would speed up the process (Hettinga & Zhang, 2018; Wadood *et al.*, 2025).

The use of multi-omics approaches has significant potential to revolutionize precision livestock farming. Multi-omics, which is the integration of different molecular pathways, is the design for linking a genotype to a particular phenotype through protein intermediates. Thus, it allows for improving selection, early diagnosis, and specific management of livestock. This, in turn, is associated with improved resource use, animal health, and environmental quality.

In addition, decisions involving omics can help limit the dependency on antibiotics in animals, enhance their reproductive efficiency, and thus, feed use is more effective. Not to mention that these advantages are not just theoretical, for instance, studies like Liu *et al.*, (2022) and Sun *et al.* (2020) provide live examples, showing that the applications are in practice.

Nonetheless, it is obligatory to conduct not only research but also the infrastructure, training, and economic feasibility studies to make ideas work. Through complicated situations like this, omics adoption in the smallholder and developing countries' systems seems to be harder. This, therefore, necessitates the availability of user-friendly technologies and capacity-building projects. For instance, in MSTN gene-edited cattle, a 2025 study showed that gut microbiota alterations significantly influenced host fatty acid metabolism, as revealed by a combined transcriptome and metabolome analysis, highlighting omics' role in functional validation of gene editing (Zhao *et al.*, 2025).

From the detailed overview of Tables 1 to 6 and Figures 1 to 5, we conclude that there are crucial directions for the progress of multi-omics research in dairy cattle. The first one is the urgent need to broaden the breed diversity by introducing non-Holstein and indigenous breeds, which will multiply the global applicability and inclusiveness of research output. Second, the studies should focus on the integration of the models in frameworks development that go beyond the duo models into more complex, multi-layered systems biology approaches that can better depict the different factors involved in trait regulation. The third factor, which is the concentration on increasing sample diversity, serves agricultural biotechnology with the essential techniques of tissue-based, time-series, and environmental-contextualized sampling strategies that would assure more comprehensive and biologically relevant datasets. The fourth point is that the validation should be given more emphasis, including both field tests and rechecking of biomarkers and predictive models found in different populations and environments (Sigdel *et al.*, 2019; Algharib *et al.*, 2024). Fifth, proactive financial injection in the construction of attributed livestock-specific, user-friendly, and open-access multi-omics platforms is indispensable for the democratization of data analysis and the support of reproducibility. For instance, the topics that the researchers should address in future research should be about the traits associated with the sustainability of the animals, such as resilience to stress from heat, the ability to resist infections, and the level of methane gas released, thus bringing the targets of the genetic enhancement to the level of environmental and climatic global concerns.

In conclusion, future research should prioritise: (1) expanding multi-breed representation, especially indigenous and dual-purpose cattle; (2) developing unified omics integration pipelines; (3) investing in time-series and tissue-specific sampling; and (4) validating findings under commercial field conditions to ensure practical translation.

Conclusion: To summarize the literature analyzed, a shift towards integrative and data-rich methodologies in dairy science is evident. Genomics remains the primary focus, yet the inclusion of transcriptomic, proteomic, metabolomic, and microbiomic data elements provides a more holistic and precise understanding of dairy cattle phenotypes. The collaboration, standardization, and rational use of these strong technologies in developing sustainable, resilient, and productive systems are the future of dairy research.

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Cross-disciplinary collaboration, accessible data infrastructure, and breed-inclusive policies will be pivotal to realising the full potential of multi-omics in advancing sustainable dairy production.

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