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REVIEW

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(final version of manuscript before type-setted PDF)

Transcriptome and metabolome integration in sheep meat quality: a systematic review of molecular mechanisms, key pathways, and candidate biomarkers

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Abstract. Sheep meat quality, including tenderness, intramuscular fat content, flavour, and colour, is determined by complex transcriptional and metabolic networks in muscle tissue. While integrated omics profiling is increasingly used to investigate these mechanisms, the available literature has not yet been systematically synthesised. This review examined 27 studies (2016–2026) employing transcriptomic and metabolomic analyses to investigate sheep muscle characteristics and identify genes, metabolites, and pathways reported in multiple studies rather than relying on results from individual works. The most common findings involved peroxisome proliferator-activated receptor (PPAR) signalling (6 studies), glycolysis, and oxidative phosphorylation. However, the same genes were rarely identified in different studies. Only *PPARGC1A*, *FABP4*, and *PLIN1* were reported in more than one study, whereas most genes were identified in only a single study. Metabolites showed greater consistency

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across studies, with arachidonic acid and purine nucleotides each reported in three studies. Only one article included functional validation beyond gene expression analysis. Although lipid metabolism, energy regulation, and amino acid pathways were associated with meat quality traits, including intramuscular fat (IMF) deposition and flavour, the evidence remains insufficient to establish specific genes or metabolites as reliable biomarkers. The field is limited by inconsistent reporting thresholds and a lack of data sharing. Further progress requires standardised criteria for identifying significant changes, full data availability, and functional experiments. Without these steps, no gene or metabolite can be considered a validated biomarker of sheep meat quality.

Keywords: intramuscular fat, metabolomics, multi-omics integration, PPAR signalling, sheep meat quality, transcriptomics

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Introduction

Sheep meat is a globally significant protein source, and consumer demand has shifted from simple availability toward consistent tenderness, flavour, and nutritional composition, all of which are considered attributes of meat quality and operationally defined through measures such as intramuscular fat (IMF) content, shear force, pH decline, water-holding capacity, colour stability, and the profile of volatile and non-volatile flavour compounds (Anwar et al., 2025; Ma et al., 2025; Liang et al., 2026). Traditional approaches to improving these traits relied on phenotypic selection, carcass grading, and single-candidate-gene studies, which can identify associations but rarely elucidate the underlying regulatory mechanism linking genotype, gene expression, metabolite flux, and the final phenotype (Li et al., 2025; Wang et al., 2026).

Multi-omics integration most commonly involving the joint analysis of transcriptomic (RNA-seq) and metabolomic (GC-MS, LC-MS, or UPLC-MS/MS) data from the same muscle samples, has been proposed as a more mechanistic alternative. This approach is based on the premise that differentially expressed genes (DEGs) provide hypotheses about regulatory mechanisms, while differentially abundant metabolites (DAMs/DMs) give information about downstream metabolic changes. Furthermore, genes and metabolites that co-vary in the same samples are more likely to lie within the same biological pathway than findings from either omics layer alone (Rohart et al., 2017). One example is the multivariate integrative omics framework Data Integration Analysis for Biomarker discovery using Latent components (DIABLO), implemented in the mixOmics package and used in several of the included studies (e.g., Liang et al., 2026). Over the past decade, this approach has been applied in a wide range of sheep breeds and rearing backgrounds, including Chinese breeds (Hu, Tan, Dolang, Minxian black fur, Hanzhong), Tibetan-plateau ecotypes, crossbred populations, and a variety of dietary

or environmental interventions, such as herbal and probiotic feed additives, rumen-protected glucose saline-alkali rearing, mycotoxin or parasite challenge, and cold-season housing.

To our knowledge, no previous review has systematically assessed which genes, metabolites, and pathways proposed as key regulators of sheep meat quality have been reported by more than one independent research group. Distinguishing independently replicated findings from those reported in individual studies is important for two practical reasons. First, breeding and nutritional intervention programmes targeting candidate biomarkers need to establish whether a candidate gene has independent supporting evidence. Second, the multi-omics literature in livestock science has a recognised tendency to produce descriptive lists of differentially expressed genes (DEGs) and differentially abundant metabolites (DAMs), with limited functional follow-up (Wang et al., 2026). Quantifying the extent to which specific genes and metabolites are consistently reported across studies provides a way to examine this concern specifically in the context of sheep meat quality. The novelty of the present review lies in this replication audit rather than in the topic itself. Previous multi-omics review articles in small ruminants have summarised which mechanisms have been proposed and which technologies are available. To our knowledge, no previous review has applied a pre-specified replication rule at the gene, metabolite, and pathway levels, reported replication rates quantitatively, or documented shared senior authorship between included studies to avoid counting findings from the same research group as independent replications. This review therefore provides an explicit and reproducible evaluation of the extent to which specific molecular findings in this literature are corroborated, together with the criteria used to for this assessment.

Using the population, intervention, comparison, outcome (PICO) format, the population comprised sheep (*Ovis aries*) from which skeletal muscle or other muscle-related tissues were sampled. The factors investigated included breed, diet, environmental conditions, and developmental stage, with both transcriptomic and metabolomic analyses performed to investigate their associations with muscle characteristics and meat quality. The comparator was the within-study control/contrast group, while the outcome included any meat-quality-relevant phenotype such as tenderness, IMF content, flavour, colour, and volatile and non-volatile flavour compounds, as well as DEGs, DAMs, and pathways reported to be associated with these traits. The present review had three objectives: (1) to identify and map all sheep studies meeting this PICO definition published between 2016 and 2026; (2) to identify which genes, metabolites, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were reported in more than one study; and (3) to characterise the methodological heterogeneity in terms of

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sample sizes, statistical thresholds, analytical platforms, and validation approaches, and to assess how these differences affect confidence in the reported findings.

Materials and methods

This review follows the PRISMA 2020 reporting guidelines, where applicable (Page et al., 2022), but explicitly reports its deviations: the review was conducted by a single reviewer, without a recorded protocol, a second independent screener, and a specified risk-of-bias assessment tool. Although PRISMA recommends independent screening by at least two reviewers, this was not possible in the present review.

Definitions and terminology: In the current review, 'transcriptome' refers to the complete set of RNA transcripts present in a sample, while 'transcriptomic analysis' refers to genome-wide expression profiling, which was performed using RNA sequencing in all included studies. 'Metabolome' refers to the set of low-molecular-weight metabolites measured in a sample by GC-MS, LC-MS or UPLC-MS/MS. 'Differentially expressed gene (DEG)' is a transcript reported by the original authors as significantly different between groups according to the statistical threshold used in the respective study. The terms 'differentially abundant metabolite (DAM)" and 'differential metabolite (DM)' are used interchangeably in the primary literature. For consistency, DAM is used throughout this review for any metabolite reported as significantly different between groups, while DM is retained only when describing the terminology used by the original authors. 'Metabolite feature' is an unannotated mass-spectrometric signal defined by its mass-to-charge ratio and retention time that has not been assigned a chemical identity. Feature counts are therefore not equivalent to counts of identified metabolites, and this distinction is maintained when comparing DAM counts across studies.

Search strategy

Two systematic queries were conducted in PubMed: (1) 'sheep transcriptome metabolome meat quality' (27 records, 2016 to May 2026) and (2) 'sheep meat quality transcriptome metabolome muscle' (22 records, 17 of which were not captured by the first query). Five targeted follow-up searches were carried out to verify specific breed and study names identified during full-text screening (Minxian black fur sheep, Dolang sheep, Tibetan sheep spermatogenesis/high-altitude, Tianhua/Gansu Alpine sheep, gut-tract/semitendinosus sheep-goat), yielding seven additional records. The Consensus academic search engine (including Semantic Scholar, Scopus, and PubMed) was used for independent cross-checking with the query 'sheep muscle transcriptome metabolome integration meat quality'. The search returned 20 matches (10 displayed) and identified no additional records beyond those retrieved

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through the targeted PubMed searches, apart from one study on the gut tract and semitendinosus, which was subsequently independently confirmed and retrieved through PubMed. In total, 51 unique records were identified through database searches. One additional study (S20) was provided directly as a full-text document rather than retrieved by database searches. It has since been identified and verified as a published, peer-reviewed article (Ma et al., 2025) and is referred to as S20 throughout the review. It is distinct from the other study by Ma et al. included in the review (S21; Ma et al., 2024). The provisional flag indicating that S20 was independently unverified in the previous version has therefore been removed. The search period was specified *a priori* as 2016–2026. Records published between 2016 and 2019 were retrieved and screened, but none met inclusion criterion 2, as paired transcriptomic and metabolomic analyses of sheep muscle have been reported in this literature only since 2020. No eligible study was excluded on the basis of publication date. The 2020–2026 publication period of the included studies therefore reflects the screening results, rather than a restriction imposed on the search period.

Inclusion criteria

Studies were included if they (1) used *Ovis aries* (sheep) as the study species; (2) performed both transcriptomic profiling (typically RNA-seq) and metabolomic profiling (GC-MS, LC-MS, or UPLC-MS/MS) on the same or matched sample sets; (3) sampled skeletal muscle or a tissue directly relevant to meat quality; and (4) reported at least one outcome relevant to meat quality (tenderness, IMF, shear force, pH, colour, flavour compounds, or DEGs and DAMs explicitly framed as relevant to these results). Studies that satisfied criteria 1, 3 and 4 but included only one type of omics data, or provided paired omics data for tissues other than muscle, were not included in the core set. Instead, they were retained separately as contextual evidence (Table 1, S9, S10, S19), and their contribution to gene, metabolite and pathway counts was reported separately.

Rationale for the tissue and design scope: skeletal muscle is the edible product, but several included studies examined tissues other than muscles, including intermuscular or tail fat depots and gastrointestinal segments, or combined microbial profiling with paired omics analyses of muscle. These designs were retained for three reasons. First, adipose depots are the direct site of the lipid-metabolic processes (PPAR signalling, lipolysis regulation, fatty acid biosynthesis) that determine intramuscular fat content and fatty acid composition, and the studies concerned reported muscle meat-quality phenotypes alongside the depot data. Second, gut-derived amino acids, fatty acids and microbial metabolites are the substrates in muscle metabolism, thus gut-focused studies investigate potential links between gut metabolism and the availability of meat flavour precursors. Third, excluding these studies would have removed

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the only studies addressing the emerging gut-muscle axis. To prevent this broader scope from artificially increasing the replication counts, studies that do not provide paired transcriptomic and metabolomic data from muscle or muscle-relevant tissue are classified as contextual evidence (Table 1, S9, S10 and S19). Their contributions to gene, metabolite and pathway counts are reported separately.

Exclusion criteria

Records were excluded for the following reasons: incorrect study species (cattle, pigs, rabbits, geese or crayfish; 16 records); *in vitro* cell line models rather than intact muscle tissue (1 record: a Hu sheep hepatocyte-preadipocyte lipid deposition model); outcomes unrelated to meat quality (1 record: a feed-additive study focused on immune function); and studies lacking integrated transcriptomic and metabolomic analyses (2 records: both investigating Tianhua mutton sheep genetics and rumen microbiota). Three narrative reviews and two studies applying an integrative multi-omics approach to sheep reproductive tissues (testis and epididymis) were retained separately rather than excluded entirely. These reproductive studies are discussed briefly in Section 5.6 for their methodological relevance but were not included in the final selection of 27 studies.

Data extraction

For each included study, the following data were recorded: the breed-comparison, sample size, tissue analysed, omics platforms used, numbers of DEGs and DAMs, individual genes and metabolites reported by name, their direction of change and, where available, fold changes, as well as KEGG pathways reported as significantly enriched. Data were extracted from the published abstract and, where accessible, the full text. One limitation of the extraction process should be noted: during HTML/XML-to-text conversion, italicised gene symbols were lost from several open-access articles. Consequently, in a small number of studies (e.g., S11 on Hu sheep carcass performance), the hub genes identified by the original authors could be retrieved, but the corresponding gene symbols could not be recovered from the available text. These entries are recorded as not recoverable rather than inferred.

Methodological quality assessment

A formal risk-of-bias assessment tool was not used because existing tools, such as SYRCLE, are not designed for observational multi-omics studies in livestock. Instead, sample

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size per group, DEG validation by qPCR or Western blot (which confirms expression but not function), and functional validation through knockdown, overexpression, or an in vivo intervention targeting the candidate pathway were recorded (Table 5).

Synthesis approach

Considering the heterogeneity of DEG-DAM thresholds ($|\log2FC| \geq 1$ vs. ≥ 2; nominal $P < 0.05$ vs. FDR-adjusted P-values) and platforms across studies, no meta-analysis or joint effect size was attempted. Instead, a narrative, count-based synthesis was performed, recording how many independent studies reported gene X or pathway Y. This was considered the most methodologically justifiable approach given the available data, and the synthesis was not presented as a meta-analysis. Replication criteria were pre-specified as follows: a gene, metabolite or KEGG pathway was counted as replicated when it was independently reported as differentially expressed, differentially abundant or significantly enriched in at least two included studies. Two further rules were applied. First, entries reported at class level (e.g., acylcarnitines or glycerophospholipids) were counted as replicated only when the class was named in both studies, and the tables indicated whether the same individual compound was reported in both studies. Second, because several included articles shared senior authors, they were treated as a single independent group when assessing whether findings were independently replicated. Accordingly, the numbers of studies and independent author groups are reported separately in Table 2, which identifies studies with overlapping senior authorship. Findings that met the replication criteria only when contextual-evidence studies were included (S9, S10 and S19) are identified as such throughout the text.

Results

Study selection and characteristics

Figure 1 shows the PRISMA-style study selection flow: 51 unique records identified through database searches and one additional record supplied independently of the search were screened together (n = 52). Of these, 25 were excluded at screening for the reasons given in Section 2, resulting in 27 studies included in the synthesis: 24 core studies with paired transcriptomic and metabolomic data and three studies retained as contextual evidence (S9, S10, S19). The included studies (Table 1) span 2020–2026 (median 2025) and were predominantly conducted by Chinese research groups investigating indigenous Chinese sheep breeds or crossbreeds, including Hu, Tibetan ecotypes, Tan, Dolang, Hanzhong, Dumengsa, Minxian black fur, and Small-Tail/Small-Tailed Han sheep. Most studies sampled the *longissimus dorsi* or *longissimus lumborum* muscle. Only four studies deliberately compared

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more than one functional muscle: S5 (Zhan et al., 2026; Tan sheep), S7 (Li et al., 2025; Hanzhong sheep), S18 (Wang et al., 2025; Minxian black fur sheep) and the contextual study S19 (Anwar et al., 2025; Dolang sheep). Sample sizes were small by clinical-trial standards but typical of livestock omics, with most group sizes ranging from n = 3 to n = 20. A few larger nutrition trials (S15, S8) included up to n = 30 per group.

Transcriptomic findings

Twenty of the 27 included studies reported a transcriptomic analysis with a specific DEG count. Six of the remaining seven studies (S6, S9, S10, S17, S23, S26) did not report a DEG count in the available text. The seventh, S19 (Anwar et al., 2025; Dolang sheep) used untargeted metabolomics without a transcriptomic component and therefore did not meet inclusion criterion 2. It was reclassified as contextual evidence, alongside S9 and S10, and excluded from the 24-study core set and all gene- and pathway-level counts. Its metabolite data were retained only where explicitly identified as contextual (Tables 1 and 3). Reported DEG counts ranged from 46 (Zhang et al., 2022) to 3 479 (Zhang et al., 2025a), reflecting differences between the comparisons, from minor dietary changes to substantial dietary protein restriction, as well as the variation in DEG thresholds described in Methods.

At the level of individual genes reported across studies, rather than raw DEG counts, the evidence was considerably sparser than the raw counts suggested (Table 2; Figures 2 and 6). A total of 41 gene symbols were catalogued from the available text. Of these, only three were mentioned in more than one study: *PPARGC1A* (PGC-1α), reported in three studies (S1, S14, S22; Kong et al., 2023; Zhang et al., 2025b; Liang et al., 2026), although S14 and S22 share senior authors, meaning that these studies represented two independent author groups rather than three; *FABP4*, reported in two studies (S18, S20; Ma et al., 2025; Wang et al., 2025); and *PLIN1*, reported in the same two studies. All other genes, including well-characterised loci related to muscle-biology such as *CPT1A*, *ABCA1*, *METTL21C*, *WFIKKN2*, *MYL2*, *MYH8*, *MYH2*, *TPM1*, *AOC1*, *HDC*, *LAPTM5*, *PER3*, and *HSPA8*, were each reported in a single study (Table 2).

The most frequently occurring pathway-level finding was enrichment of the PPAR signalling pathway, reported as significantly enriched in six studies (S3, S4, S11, S18, S20, S25), covering breed comparisons, dietary interventions and comparisons between anatomical sites (Table 4; Figure 3). Glycolysis/gluconeogenesis and oxidative phosphorylation were the next most frequently reported pathways, with four studies each (S5, S12, S20, S27 and S1, S11,

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S14, S18, respectively). S19 was excluded from the glycolysis/gluconeogenesis count following its reclassification as contextual evidence. Nine other pathways were each reported in three studies: purine metabolism, fatty acid biosynthesis, arachidonic acid metabolism, the pentose phosphate pathway, glycine/serine/threonine metabolism, arginine and proline metabolism, glycerophospholipid metabolism, AMPK signalling and PI3K-Akt signalling.

Metabolomic findings

Reported DAM/DM counts ranged from 30 (Liang et al., 2026) to 823 (Zhang et al., 2025b), reflecting differences in thresholds and analytical platforms rather than necessarily biological differences in the magnitude of metabolite changes. At the level of individually listed metabolites with cross-study confirmation (Table 3; Figure 4), arachidonic acid was the most consistently recorded metabolite, with higher levels reported in the higher-quality or higher-PUFA group in three studies : S8, the resveratrol/HMB study; S20 (Ma et al., 2025); and S27 (Wu et al., 2020). In the resveratrol/HMB study, arachidonic acid was also proposed as a hub in a flavour-related pathway, released from membrane phospholipids such as PC(38:5) through a phospholipase-mediated step. Purine nucleotides (ADP, GMP, 3′-AMP, adenine, guanine) were another group of metabolites reported in three studies : S13 (Bai et al., 2025), S14 (Zhang et al., 2025b), and S16 (the purine-content study). Carnosine and anserine, histidine-derived dipeptides associated with meat antioxidant capacity and umami-related flavour, were each reported in two studies (Kong et al., 2023; Li et al., 2025), with higher levels in the more tender or higher-quality muscle or group in both cases. L-valine (S15, S20) and the glycerophospholipid class (S18, S23) were each reported in two core studies, whereas acylcarnitines (S7, S19) and D-mannose 6-phosphate (S19, S23) were reported in two studies only when the contextual study S19 was included; each was therefore supported by only one core study. Applying the replication criteria defined in the Methods section, six of the ten catalogued metabolite entries were replicated within the core set, while two additional entries were replicated only when contextual evidence was included. Linoleic acid and the oxylipins 12-hydroperoxyeicosatetraenoic acid (12-HPETE) and prostaglandin D2 (PGD2) were single-study findings, as were the remaining metabolites discussed in individual articles.

Integrated transcriptome-metabolome findings

A limited number of studies reported specific associations between gene expression and metabolite levels. These included associations of METTL21C with carnosine and AOC1 with carnosine, L-glutamate and L-proline (Kong et al., 2023); FABP4 and PLIN1 with arachidonic

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acid, and PCK1 with groups of flavour-related metabolites, essential amino acids and polyunsaturated fatty acids (Ma et al., 2025); and AGPAT1 with sn-glycerol-3-phosphoethanolamine/glycerol-3-phosphate (Xu et al., 2023). (Figure 4; full list in the accompanying data file). No specific gene–metabolite association was reported in more than one independent study. Thus, each association shown in Figure 4 was supported by a single study, indicating limited independent evidence for specific gene–metabolite relationships compared to the more consistent replication observed at the pathway level.

Subgroup patterns: muscle anatomical site, breed and diet, and an emerging gut–muscle axis

The four studies that directly compared multiple muscles within the same animals (S5, S7, S18 and the contextual study S19) consistently found that more oxidative (slow-twitch-dominant) muscles had higher levels of flavour-related metabolites, including anserine, carnosine, and most amino acids, while glycolytic (fast-twitch-dominant) muscles, such as the *longissimus dorsi*, generally showed the opposite pattern. An opposite pattern was reported for glycerophospholipid levels in at least one study (S18). These anatomical differences were more consistent across studies than most breed or dietary effects. However, only four studies directly compared multiple muscles, and one of them (S19) provided metabolomic data only.

A more recent hypothesis, proposed in three studies published in 2025 (S2, S9, S10), suggests that gut microbiota composition and gene expression in specific gastrointestinal segments may influence the amino acid and fatty acid pools that ultimately affect muscle flavour and quality. This potential gut–muscle axis remains preliminary as all three studies were published in 2025, and at least two used similar approaches involving RNA-seq based on multiple gastrointestinal tissues. Independent confirmation by other research groups is therefore required.

The proposed roles of other genes as key regulators are likewise based on differential expression and correlation or co-expression network analyses, including weighted gene co-expression network analysis (WGCNA), Data Integration Analysis for Biomarker discovery using Latent variable approaches for Omics studies (DIABLO) and Pearson correlation, rather than experimental manipulation.

Discussion

Summary of principal findings

Three pathway-level patterns were repeatedly identified in the 27 included studies, spanning six years and a wide range of Chinese sheep breeds, rearing environments and diets:

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lipid storage and PPAR signalling, AMPK-linked energy metabolism, and amino-acid metabolism related to flavour- and antioxidant-associated compounds. However, replication was very limited at the level of individual genes and specific gene–metabolite correlations. Of the 41 catalogued genes, only 3 were reported by more than one study, and *PPARGC1A*, the most consistently reported of these genes, was reported by only two independent author groups; Among the 13 specific gene–metabolite correlation pairs identified across the studies, none was reported by more than one independent group.

Interpreting the consistent pathways

Lipid metabolism/IMF

The studies identified several aspects of lipid metabolism, including PPAR signalling, regulation of lipolysis in adipocytes, and fatty acid biosynthesis. This was the most consistently reported pathway in the literature, with supporting evidence from breed comparisons (S11, S20, S25), anatomical-site comparisons (S18) and dietary interventions (S3, S4). *PLIN1* and *FABP4*, the two genes with the strongest, although still modest cross-study support, both belong to this pathway, aligning with their established roles in intracellular fatty-acid transport and lipid-droplet maintenance, respectively. However, this interpretation cannot be directly tested in sheep because the available evidence is limited to the two supporting studies.

AMPK-linked energy metabolism

The articles contained information on several aspects of AMPK-linked energy metabolism, including glycolysis/gluconeogenesis, oxidative phosphorylation, the pentose phosphate pathway, and AMPK signalling. These pathways were examined in different types of comparisons, including dietary interventions (S5, S12, S27), anatomical comparisons (S18), genetic comparisons (S20), and pathogen- or toxin-related challenges (S12). In S12, aflatoxin B1 and *Eimeria* challenge specifically disrupted the HIF-1α/HK2/glycolysis axis. The occurrence of these pathways in such diverse contexts suggests that they may represent a general response to stress and energy status rather than a mechanism specific to meat quality. However, this interpretation cannot be verified from the available data because none of the 27 studies was designed to directly test pathway specificity.

Amino-acid metabolism and flavour/antioxidant compounds

The studies identified several aspects of amino-acid metabolism, such as arginine-proline metabolism, glycine/serine/threonine metabolism, and taurine/hypotaurine metabolism, as well as metabolites such as carnosine, anserine, and arachidonic-acid metabolites. Higher

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carnosine and anserine levels were frequently found in more tender or higher-quality muscle (S7, S22), consistent with the known antioxidant properties and possible flavour-related roles of these histidine-derived dipeptides. However, the available evidence could not distinguish whether these compounds were directly associated with meat quality or represented downstream markers of oxidative muscle fibre type, as both supporting studies also linked them to muscle fibre type, or both.

Relating the molecular findings to measured meat-quality traits.

The pathway-level findings can be related to the traits that define carcass value, although the extent of the available evidence differs between traits. The relationship is the most direct for intramuscular fat and fatty acid composition: PPAR signalling, regulation of lipolysis in adipocytes, and fatty acid biosynthesis were enriched in the studies comparing high- and low-IMF or high- and low-carcass-performance groups (S11, S20, S24, S26). The two replicated genes in this group, *FABP4* and *PLIN1,* are involved in intracellular fatty acid transport and lipid-droplet stabilisation, respectively. Higher expression of these genes in the high-IMF or higher-meat-quality group was consistent with their involvement in intracellular lipid storage, but the available data did not allow to establish a direct relationship with triacylglycerol storage in muscle.

For tenderness and post-mortem pH decline, the relevant pathways involve glycolysis and mitochondrial energy metabolism. Animals with muscles characterised by a more glycolytic profile have greater glycogen stores and glycolytic capacity, produce lactate more rapidly after slaughter, and reach a lower final pH, which in turn affects water-holding capacity, drip loss and shear force. This provides a physiological context for the recurrent enrichment of glycolysis/gluconeogenesis and oxidative phosphorylation pathways (S1, S5, S11, S12, S14, S18, S20, S27) and for the fibre-type genes identified in the multi-muscle comparisons: *MYH2* and *TPM1* were up-regulated in the more tender *longissimus dorsi* in S7, while *MYL2, TNNC1, TNNI1* and *TPM3* were associated with oxidative muscles in S18. Purine metabolism is also relevant to this interpretation, since post-mortem nucleotide degradation is related to the energy state of the muscle at slaughter (S13, S14, S16). It should be noted, however, that only a minority of the included studies reported shear force or final pH alongside the omics data, thus this linkage is based more on established muscle physiology than on within-study correlations.

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Regarding flavour, the amino-acid and lipid-derived precursor pathways link the molecular findings to the formation of flavour-related compounds. Carnosine and anserine, which showed elevated levels in the more tender or higher-quality muscle in S7 and S22, contribute to antioxidant capacity and may also influence umami-related taste. Meanwhile, free amino acids generated through glycine/serine/threonine and arginine/proline metabolism are precursors for Maillard and Strecker reactions that contribute to cooked-meat aroma. Arachidonic acid, released from membrane phospholipids through glycerophospholipid turnover, is a precursor of oxylipins and short-chain aldehydes involved in species-characteristic mutton aroma (S8, S18, S20, S25, S27). Colour stability was the least frequently assessed trait. It depends on myoglobin content and the antioxidant and reducing capacity of muscle, which may link it to the taurine/hypotaurine and pentose phosphate pathways through NADPH supply, as reported in S11, S20, S22, and S23. However, none of the included studies measured metmyoglobin accumulation or colour stability during refrigerated display. The limited assessment of specific meat-quality features restricts the extent to which these molecular findings can be related to specific traits or translated into selection or feeding strategies.

Candidate biomarkers and validation status

Tables 2 and 3 list every gene and metabolite that could be verified, together with the number of supporting studies. A single-study finding was not considered sufficient to classify a gene or metabolite as a validated biomarker. According to the replication criteria defined in the Methods, 38 of the 41 catalogued genes were reported in only one study, whereas 8 of the 10 catalogued metabolite entries were reported by at least two studies (6 within the core set, and 2 when contextual evidence was included). Only 1 of the 27 studies provided functional validation (Table 5). The three genes and the replicated metabolites have been reported in more than one study, but this level of replication remains limited. *PPARGC1A*, the most consistently identified gene in this literature, was reported by only two independent author groups and showed an inconsistent direction of change in the three studies, in which it was discussed. These candidates should therefore be treated as hypotheses for validation rather than as established biomarkers.

Comparison with existing reviews

Three reviews identified during the search provided useful context rather than primary evidence. Wang et al. (2023a) reviewed multi-omics approaches (transcriptomics, proteomics, and metabolomics) in sheep and goat meat and concluded that the field was still transitioning

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from descriptive omics toward validated biomarkers, a conclusion supported by the present gene- and metabolite-level replication analysis, which provides a specific, measured number rather than a general impression. Li et al. (2024a) reviewed pan-omics in sheep across growth, reproduction, disease resistance, and product quality traits, with a broader scope than meat parameters, and did not perform a cross-study replication analysis. Wang et al. (2023b) examined Chinese herbal medicine feed additives in relation to mutton flavour, based on evidence from 2014–2024, and proposed epigenetic and microbial mechanisms. The gut–muscle axis emerging from the 2025 primary literature was consistent with, and was partly anticipated by, the microbial mechanisms discussed in that review.

Methodological heterogeneity and limitations of the available evidence

In addition to the heterogeneity in thresholds and analytical platforms described in the Methods, the primary literature has three further limitations. First, the literature is geographically and institutionally concentrated. Nearly every included study originates from a small number of Chinese institutions, including the Lanzhou Institute of Husbandry and Pharmaceutical Sciences/CAAS, Gansu Agricultural University, and several other centres. Some of these studies have the same senior authors; for example, Zengkui Lu and Jianbin Liu are senior authors on S14, S21, S22, and the related testis study. This does not detract from the quality of the individual studies, but it indicates that the consistency reflects findings generated within a relatively narrow scientific community and breed-sampling framework rather than evidence from a broader international research base. Shared senior authorship is therefore flagged in Table 2, and the number of independent author groups is reported alongside the number of studies. Second, most studies relied on *longissimus dorsi/lumborum* as the sampled muscles. Only 4 of 27 studies tested more than one anatomical muscle, limiting the evidence for mechanisms that could apply to different muscles. Third, functional validation was largely absent. Only one research group experimentally tested a candidate pathway *in vivo* (Table 5). The proposed roles of other genes as key regulators were inferred from differential expression and correlation network analyses rather than established through functional validation.

Related multi-omics studies of reproductive tissues

Two studies from the broader search (Li et al., 2024b; Zhang et al., 2024), used an essentially identical integrative transcriptome–metabolome design in sheep but addressed reproductive rather than meat-quality phenotypes. These studies were not included in the count of 27 because they addressed a distinct biological outcome from meat quality. Both studies

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independently reported AMPK signalling (Garcia and Shaw, 2017), glycolysis/gluconeogenesis, and the tricarboxylic acid (TCA) cycle as recurring pathway-level findings in sheep multi-omics studies outside the meat-quality context. This supports the interpretation that this pathway cluster represents a relatively general energy-metabolism signal across tissues rather than one specific to muscle or to meat quality.

Future directions

Four steps would improve the reliability and comparability of this literature. First, DEG/DAM reporting should follow consistent criteria. A shared minimum threshold and FDR-correction convention, similar to the Minimum Information About a Microarray Experiment and Minimum Information for Biological and Biomedical Investigations (MIAME/MIBBI) standards used in other omics fields, would make cross-study counts comparable. At present, differences in reporting criteria limit such comparisons. Second, complete data deposition is required. Most studies report only a small number of key genes in the abstract or main text, while the full DEG/DAM tables are available only in supplementary files that were not accessible. As a result, Tables 2–4 contain only the genes and metabolites that could be verified from the available study reports. Routine deposition of complete DEG/DAM tables in standard formats would allow future reviews to compile more comprehensive cross-study datasets (Tables 2–4). Third, functional validation should be expanded. Considering that 26 of the 27 included studies did not perform functional validation, the next phase of this field should include knockdown, overexpression, and CRISPR-based approaches to test the strongest identified candidates (*FABP4, PPARGC1A* and *PLIN1*) before treating them as established targets for breeding or nutritional intervention (Table 5). Fourth, single-cell and single-nucleus RNA sequencing could address an important limitation of the current evidence base. Bulk muscle is a mixture of myofibres, adipocytes, fibro-adipogenic progenitors, satellite cells, endothelium and immune cells. For this reason, a bulk DEG list cannot distinguish changes in gene expression within a cell type from changes in the proportion of that cell type. Because intramuscular fat deposition is closely related to adipogenic cell number and activity, single-nucleus data provide more specific information on the PPAR and lipolysis findings. Spatial transcriptomics and mass-spectrometry imaging could extend this approach by preserving spatial information, allowing intramuscular fat distribution and metabolite gradients to be mapped onto the fibre architecture associated with meat quality traits.

Machine learning and genomic selection could provide two routes for applying such data to prediction and breeding. Supervised models trained on paired omics and phenotype data, with proper nested cross-validation and external test sets, would allow candidate biomarker

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panels to be evaluated according to their predictive performance for tenderness or intramuscular fat rather than according to enrichment P values. However, the sample sizes in the current literature (n = 3–30 per group) are too small for this purpose, highlighting the need for multi-institutional data pooling and the standardised reporting described above. For breeding applications, the replicated candidates identified here could be used as prior information rather than as direct selection targets, for example as weighted variants in genomic prediction, or as the basis for transcriptome-wide association and expression quantitative trait locus (eQTL) analyses that connect them to genomic markers usable in selection programmes. Integrating epigenomic layers (DNA methylation, m6A modification) and rumen or gut metagenomes would additionally allow the gut-muscle axis hypothesis to be tested experimentally rather than inferred from correlations.

Conclusions

Twenty-seven studies (2020–2026) combining transcriptomics and metabolomics data related to sheep meat-quality phenotypes reported three recurring pathway-level patterns: lipid storage and PPAR signalling, AMPK-linked energy metabolism, and amino-acid-derived flavour and antioxidant metabolism, with PPAR signalling being the most consistently identified pathway (6 of 27 studies). At the gene and metabolite level, findings reported in more than one study were uncommon: only *FABP4, PLIN1* and *PPARGC1A* were reported in at least two studies, and the latter was reported in three studies representing only two independent author groups. Among metabolites, 8 of the 10 catalogued entries were reported in at least two studies (6 of them within the core set), and arachidonic acid and purine nucleotides were each reported by three studies. Even these results were not fully consistent, as illustrated by the direction of change reported for *PPARGC1A*. Only one study included functional validation in addition to expression data. The pathway-level findings can therefore be used as a basis for hypothesis generation, whereas genes and metabolites reported in at least two studies remain candidates for further validation. Single-study findings should be regarded as preliminary until independently corroborated. For the field as a whole, greater consistency in reporting thresholds, complete deposition of DEG/DAM tables, and functional verification of the small number of candidates reported in more than one study are needed to strengthen the evidence base for sheep meat-quality breeding and nutrition research.

Conflict of interest

The Authors declare that there is no conflict of interest.

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References

Anwar A., Fu X., Tang S., Liu W., Wang Y., Zhao W., Wu C., 2025a. Untargeted metabolomics analysis reveals comparative meat quality of different muscles and the impact of Saline-alkali land feeding on Dolang sheep (*Ovis aries*). BMC Genomics 26, 782, https://doi.org/10.1186/s12864-025-12008-8

Bai X., Wang L., Sun H., Sun L., An J., Fu S., Zhao M., Liu F., Ren X., Liu Z., 2025. Yeast culture supplementation improves meat quality by enhancing immune response and purine metabolism of small-tail Han sheep (*Ovis aries*). Int. J. Mol. Sci. 26, 4512, https://doi.org/10.3390/ijms26104512

Garcia D., Shaw R.J., 2017. AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. Mol. Cell 66, 789–800, https://doi.org/10.1016/j.molcel.2017.05.032

Kong L., Yue Y., Li J., Yang B., Chen B., Liu J., Lu Z., 2023. Transcriptomics and metabolomics reveal improved performance of Hu sheep on hybridization with Southdown sheep. Food Res. Int. 173, 113240, https://doi.org/10.1016/j.foodres.2023.113240

Li M., Lu Y., Gao Z., Yue D., Hong J., Wu J., Xi D., Deng W., Chong Y., 2024a. Pan-omics in sheep: unveiling genetic landscapes. Animals 14, 273, https://doi.org/10.3390/ani14020273

Li Y., Yang Y., Yu B., Gao R., Wang X., 2024b. Transcriptome and metabolome analyses reveal high-altitude adaptation mechanism of epididymis sperm maturation in Tibetan sheep. Animals 14, 3117, https://doi.org/10.3390/ani14213117

Li Z., Zheng M., Li W., Li J., Wang L., Wang S., Lu H., Zhang T., 2025. Carnosine and acyl carnitines as metabolic determinants of muscle phenotypic differences between *longissimus dorsi* and *triceps brachii* in Hanzhong sheep. Foods 14, 3289, https://doi.org/10.3390/foods14193289

Liang Y., Shi J., Cheng X., Feng M., Jiao D., Du X., Ji K., Hu S., Dong Q., Yang G., 2026. Integrative transcriptomics and metabolomics analyses reveal changes in meat quality and muscle lipid metabolism in sheep supplemented with rumen-protected glucose. Meat Sci. 110110, https://doi.org/10.1016/j.meatsci.2026.110110

Liu Y., Wang C., Li M., Yang Y., Wang H., Chen S., He D., 2025. Unveiling key genes and crucial pathways in goose muscle satellite cell biology through integrated transcriptomic and metabolomic analyses. Int. J. Mol. Sci. 26, 3710, https://doi.org/10.3390/ijms26083710

Ma K., Song J., Li D., Liu Z., Wang C., Li T., Ma Y., 2025. Insights into the differences in meat quality among different sheep breeds in the Qilian Mountains from the perspective of metabolomics and transcriptomics. Food Biosci. 63, 105693, https://doi.org/10.1016/j.fbio.2024.105693

Ma K.-Y., Song J.-J., Li D.-P., Wu Y., Wang C.-H., Liu Z.-L., Li T.-T., Ma Y.-J., 2024. Genomic structure analysis and construction of DNA fingerprint for four sheep populations. Animal 18, 101116, https://doi.org/10.1016/j.animal.2024.101116

IAS Article in Press

Page M.J., McKenzie J.E., Bossuyt P.M., Boutron I., Hoffmann T.C., Mulrow C.D., Shamseer L., Tetzlaff J.M., Akl E.A., Brennan S.E., 2022. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Revista panamericana de salud publica 46, e112, https://doi.org/10.1136/bmj.n71

Rohart F., Gautier B., Singh A., Lê Cao K.-A., 2017. mixOmics: An R package for 'omics feature selection and multiple data integration. PLoS Comput. Biol. 13, e1005752, https://doi.org/10.1371/journal.pcbi.1005752

Wang H., Liu Z., Yang H., Bai Y., Li Q., Qi X., Li D., Zhao X., Ma Y., 2025. Integrated transcriptomics and metabolomics reveal the molecular characteristics and metabolic regulatory mechanisms among different muscles in Minxian black fur sheep. BMC Genomics 26, 412, https://doi.org/10.1186/s12864-025-11607-9

Wang J., Fu Y., Su T., Wang Y., Soladoye O.P., Huang Y., Zhao Z., Zhao Y., Wu W., 2023. A role of multi-omics technologies in sheep and goat meats: progress and way ahead. Foods 12, 4069, https://doi.org/10.3390/foods12224069

Wang Z., Sha Y., Li X., Zhao H., Zhao S., Jiao T., 2026. Multi-omics analysis of amino acids and fatty acids metabolism impacts on flavor profiles in Tibetan and Hu sheep under cold-season housing conditions. J. Sci. Food Agric. 106, 2410–2423, https://doi.org/10.1002/jsfa.70348

Wu J., Yang D., Gong H., Qi Y., Sun H., Liu Y., Liu Y., Qiu X., 2020. Multiple omics analysis reveals that high fiber diets promote gluconeogenesis and inhibit glycolysis in muscle. BMC Genomics 21, 660, https://doi.org/10.1186/s12864-020-07048-1

Xu X., Liu H., Wang X., Zhang Q., Guo T., Hu L., Xu S., 2023. Evaluation of the *longissimus thoracis et lumborum* muscle quality of Chaka and Tibetan sheep and the analysis of possible mechanisms regulating meat quality. Animals 13, 2494, https://doi.org/10.3390/ani13152494

Zhan C., Li M., Li D., Li P., Zhang Q., Wu M., Zhong G., Xu X., 2026. Metabolomics and transcriptomics reveal the effects of fermented *Lycium barbarum* (Goji) berry residue on muscle nutrition and flavor quality in fattening Tan sheep. Metabolites 16, 39, https://doi.org/10.3390/metabo16010039

Zhang B., Sun Z., Yu Z., Li H., Luo H., Wang B., 2022. Transcriptome and targeted metabolome analysis provide insights into bile acids' new roles and mechanisms on fat deposition and meat quality in lamb. Food Res. Int. 162, 111941, https://doi.org/10.1016/j.foodres.2022.111941

Zhang F., Wu Z., Sa R., Zhang Y., Zhang X., Hou S., Gui L., 2025a. Effect of different Lys/Met ratios in a low-protein diet on the meat quality of Tibetan sheep: A transcriptomics-and metabolomics-based analysis. Food Res. Int. 204, 115893, https://doi.org/10.1016/j.foodres.2025.115893

Zhang L., An X., Xu Z., Niu C., Geng Z., Zhang J., Shi H., Chen Z., Zhang R., Yue Y., 2025b. Transcriptome–metabolome analysis reveals that crossbreeding improves meat quality in

JAFS Article in Press

Hu sheep and their F1-Generation sheep. Foods 14, 1384, https://doi.org/10.3390/foods14081384

Zhang M., An X., Yuan C., Guo T., Xi B., Liu J., Lu Z., 2024. Integration analysis of transcriptome and metabolome revealed the potential mechanism of spermatogenesis in Tibetan sheep (*Ovis aries*) at extreme high altitude. Genomics 116, 110949, https://doi.org/10.1016/j.ygeno.2024.110949

Table 1. Characteristics of the 27 included studies. "Ref." gives the number of the corresponding entry in the reference list; an asterisk marks an included study whose numbered reference entry is still to be inserted. "Evidence scope" indicates whether a study met inclusion criterion 2 (paired transcriptomic and metabolomic profiling on the same or matched samples; core set, n = 24) or is retained as contextual evidence (S9, S10, S19). S20 was supplied directly as a full text rather than retrieved by the database searches; it has since been verified as a published article (reference 1: Ma, K. et al., Food Bioscience 2025, 63, 105693) and is distinct from S21 (Ma et al., 2024). Each S-code is linked to its corresponding numbered reference in the Ref." column

ID	Study	Comparison	Tissue	Omics platforms	Evidence scope	Ref.
S1	Liang et al., 2026	RPG diet vs control, Dumengsa sheep	*Longissimus lumborum*	RNA-seq + GC-MS	Core sheep-specific multi-omics	2
S2	Zhao et al., 2026	Feed additive dose-response, fattening lambs	Jejunum + muscle	16S + RNA-seq + metabolomics	Core sheep-specific multi-omics	*
S3	Wang et al., 2025	Tibetan vs Hu sheep, cold housing	Muscle + VOCs	RNA-seq + UHPLC-QTOF-MS + HS-SPME-GC-MS	Core sheep-specific multi-omics	4
S4	Yang et al., 2026	Cyanidin-3-O-galactoside dose, Small-Tailed Han lambs	Muscle	RNA-seq + untargeted metabolomics	Core sheep-specific multi-omics	*
S5	Zhan et al., 2026	Fermented goji berry residue, Tan sheep	4 muscles (LD focus)	RNA-seq + LC-MS	Core sheep-specific multi-omics	10
S6	Li et al., 2025	3 Tibetan sheep ecotypes (PT/VT/EL)	Muscle	RNA-seq + flavour metabolomics	Core sheep-specific multi-omics	*
S7	Li et al., 2025	*Longissimus dorsi* vs *triceps brachii*, Hanzhong sheep	2 muscles	RNA-seq + metabolomics	Core sheep-specific multi-omics	5
S8	Chen et al., 2025	Resveratrol+HMB, Tibetan sheep	Intermuscular fat	RNA-seq + lipidomics	Core sheep-specific multi-omics	*
S9	Chen et al., 2025	Gut-site metabolome vs LD muscle, Meigu goat/Liangshan sheep	10 gut sites + LD muscle	RNA-seq + untargeted metabolomics	Contextual evidence	*
S10	Chen et al., 2025	GI-tract transcriptome vs semitendinosus, sheep/goat	Gut + ST muscle	RNA-seq + targeted AA/FA/VOC profiling	Contextual evidence	*

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ID	Study	Comparison	Tissue	Omics platforms	Evidence scope	Ref.
S11	Zhang et al., 2025	High vs low carcass performance, Hu sheep	*Longissimus dorsi*	RNA-seq + LC-MS	Core sheep-specific multi-omics	*
S12	He et al., 2025	AFB1/*Eimeria ovinoidalis* challenge	Muscle	RNA-seq + metabolomics	Core sheep-specific multi-omics	*
S13	Bai et al., 2025	Yeast culture supplementation, Small-Tail Han sheep	*Longissimus dorsi*	RNA-seq + metabolomics	Core sheep-specific multi-omics	16
S14	Zhang et al., 2025	HHxHu vs DorsetxHu vs SouthdownxHu	*Longissimus dorsi*	RNA-seq + LC-MS	Core sheep-specific multi-omics	12
S15	Zhang et al., 2025	Dietary Lys/Met ratio, Tibetan sheep	*Longissimus dorsi*	RNA-seq + untargeted metabolomics	Core sheep-specific multi-omics	11
S16	Han et al., 2025	High vs low purine content, Australian WhitexHan hybrids	Muscle	RNA-seq + metabolomics + mouse validation	Core sheep-specific multi-omics	18
S17	Han et al., 2025	High vs low unsaturated fatty acid, lambs	*Longissimus dorsi*	RNA-seq + untargeted metabolomics	Core sheep-specific multi-omics	*
S18	Wang et al., 2025	*Biceps femoris* vs *longissimus dorsi* vs infraspinatus, Minxian black fur sheep	3 muscles	RNA-seq + UPLC-MS/MS	Core sheep-specific multi-omics	14
S19	Anwar et al., 2025	Leg vs loin; saline-alkali vs normal rearing, Dolang sheep	2 muscles	Untargeted LC-MS metabolomics only (no transcriptome)	Contextual evidence	3
S20	Ma et al., 2025	Gansu Alpine fine-wool vs Tianhua mutton sheep	*Longissimus dorsi*	RNA-seq + LC-MS/MS (targeted+untargeted)	Core sheep-specific multi-omics	1
S21	Ma et al., 2024	Dorper vs Tan sheep, multi-tissue	Brain, liver, muscle, gut	RNA-seq + gut metabolome	Core sheep-specific multi-omics	*
S22	Kong et al., 2023	SouthdownxHu vs HuxHu	*Longissimus dorsi*	RNA-seq + GC-MS + LC-MS	Core sheep-specific multi-omics	13
S23	Xu et al., 2023	Chaka vs Tibetan sheep	*Longissimus thoracis et lumborum*	RNA-seq + metabolomics	Core sheep-specific multi-omics	17
S24	Zhang et al., 2022	Bile-acid supplementation, lambs	Fat depots	RNA-seq + targeted metabolome	Core sheep-specific multi-omics	8

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ID	Study	Comparison	Tissue	Omics platforms	Evidence scope	Ref.
S25	Chen et al., 2022	Southdown/Suffolk/Hu sires x Hu dams	*Longissimus dorsi*	RNA-seq + GC-MS + LC-MS	Core sheep-specific multi-omics	*
S26	Zhang et al., 2022	High vs low IMF, Tan sheep	Muscle	RNA-seq + widely-targeted metabolomics	Core sheep-specific multi-omics	*
S27	Wu et al., 2020	High-fibre/low-protein vs low-fibre/high-protein forage, twin lambs	Muscle	16S + RNA-seq + metabolome + fatty-acid profiling	Core sheep-specific multi-omics	15

Table 2. Named genes with cross-study replication status: 3 of 41 genes are reported by two or more studies. Superscript "a" marks studies that share senior authorship and are therefore not independent; S14 and S22 share senior authors, so *PPARGC1A* is reported by three studies but by only two independent author groups.

Gene	Full name	N studies	Study IDs	Direction (per study)	Pathway
PPARGC1A	PGC-1α peroxisome proliferator-activated receptor gamma coactivator 1-alpha	3 studies / 2 independent groups	S1, S14a, S22a	Up (S1, S22); down (S14); direction not consistent	PPAR signalling; mitochondrial biogenesis
FABP4	Fatty acid binding protein 4	2	S18, S20	Up (S20); listed without a stated direction (S18)	PPAR signalling; lipolysis regulation
PLIN1	Perilipin 1	2	S18, S20	Up (S20); listed without a stated direction (S18)	Regulation of lipolysis in adipocytes
CPT1A	Carnitine palmitoyltransferase 1A	1	S1	Down	Fatty acid β-oxidation
ABCA1	ATP-binding cassette transporter A1	1	S1	Down	Cholesterol/lipid efflux
METTL21C	Methyltransferase like 21C	1	S22	Up	Muscle protein degradation
WFIKKN2	Follistatin/kunitz domain protein 2	1	S22	Down	Myostatin inhibition
AOC1	Amine oxidase, copper-containing 1	1	S22	Up	Histidine/arginine-proline metabolism
HDC	Histidine decarboxylase	1	S22	Up	Histidine metabolism
MYL2	Myosin light chain 2	1	S18	Hub gene, high in oxidative muscle	Muscle fibre type
MYH8	Myosin heavy chain 8	1	S18	High in glycolytic muscle	Muscle fibre type

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TNNC1, TNNI1, TPM3	Troponin C1/I1, Tropomyosin 3	1 each	S18	High in oxidative muscle	Muscle fibre type
MYH2	Myosin heavy chain 2	1	S7	FC = 2.92, up in tender LD	Muscle fibre type
TPM1	Tropomyosin 1	1	S7	FC = 2.15, up in tender LD	Muscle fibre type
ALDH6A1, BCKDHB, SLC16A7, LDHB	(see Table; amino-acid/glycolysis enzymes)	1 each	S18	WGCNA hub genes, flavour-AA correlated	Amino acid catabolism / glycolysis
LAPTM5, XDH, HPRT	Autophagy/purine enzymes	1 each	S16	LAPTM5 negative regulator; XDH/HPRT downstream	Purine metabolism
AK4, RRM2	Purine-pathway enzymes	1 each	S13	Altered, direction not specified in abstract	Purine metabolism
CPT1B, GOT1	β-oxidation/transaminase	1 each	S2	Up	Fatty acid oxidation / AA synthesis
PER3, EFHB	Circadian/calcium-signalling genes	1 each	S14	PER3 down	Circadian/calcium signalling
HSPA8, ACSL6, MAP1LC3B	Stress/lipid/autophagy genes	1 each	S24	Correlated with tail-fat phenotype	Ferroptosis; fatty acid biosynthesis
AGPAT1	Glycerophospholipid enzyme	1	S23	Up	Glycerophospholipid metabolism
PIK3R3, LIPE, ADIPOQ	Thermogenesis/lipolysis genes	1 each	S3	PIK3R3 down; LIPE, ADIPOQ up	Thermogenesis; lipolysis
ADRB3	Adrenergic receptor β3	1	S20	Up	Beige-adipose/lipolysis regulation

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Table 3. Named metabolites with cross-study replication status. A metabolite was counted as replicated when it was independently reported in at least two included studies (Methods). Superscript "c" marks a contextual-evidence study (S19); entries whose second supporting study is S19 are supported by one core study only. Class-level entries (acylcarnitines, glycerophospholipids) are matched at class rather than identical-compound level. Of the 10 entries, 6 are replicated within the 24-study core set, 2 are replicated only when contextual evidence is included, and 2 are single-study findings.

Metabolite	Class	N studies	Study IDs	Pattern
Arachidonic acid	PUFA (C20:4n6)	3	S8, S20, S27	Up in higher-quality/higher-PUFA groups in all 3
Purine nucleotides (ADP, GMP, 3'-AMP, adenine, guanine)	Nucleotides	3	S13, S14, S16	Central to purine-content and AMPK-pathway phenotypes
Carnosine	Dipeptide	2	S7, S22	Higher in tender/higher-quality muscle (FC = 1.07 in S7)
Anserine	Dipeptide	2	S7, S22	Higher in tender/higher-quality muscle (FC = 1.04 in S7)
Acylcarnitines	Carnitine conjugates	2 (1 core + S19 contextual)	S7, S19c	Higher in tougher muscle (S7); up under saline-alkali rearing (S19, different context)
L-valine	BCAA	2	S15, S20	Up in both higher-quality/intervention groups
D-mannose 6-phosphate	Sugar phosphate	2 (1 core + S19 contextual)	S19c, S23	Hub metabolite in both
Glycerophospholipids / sn-glycerol-3-phosphoethanolamine	Glycerophospholipid	2	S18, S23	Same class; identical compound not matched
Linoleic acid	PUFA (C18:2n6)	1	S27	Up with high-fibre diet
12-HPETE, PGD2	Oxylipins	1	S8	Proposed AA-derived flavour mediators

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Table 4. KEGG pathways recurring across the included studies. Counts refer to the 24-study core set; the contextual study S19 is not counted

Pathway	N studies	Study IDs	Relevance
PPAR signalling pathway	6	S3; S4; S11; S18; S20; S25	Intramuscular fat deposition and lipid storage/oxidation balance
Glycolysis / Gluconeogenesis	4	S5; S12; S20; S27	Energy-substrate partitioning; links to post-mortem pH decline and tenderness
Oxidative phosphorylation	4	S1; S11; S14; S18	Mitochondrial energy metabolism; oxidative vs glycolytic fibre phenotype
AMPK signalling pathway	3	S14; S18; S20	Central energy-sensing pathway linking glucose/lipid/protein metabolism
Arachidonic acid metabolism	3	S8; S18; S25	Flavour-precursor (oxylipin) generation from membrane phospholipids
Arginine and proline metabolism	3	S11; S14; S22	Amino acid metabolism linked to carnosine/related dipeptide synthesis
Fatty acid biosynthesis	3	S4; S20; S24	Lipid accretion and fatty acid profile of muscle
Glycerophospholipid metabolism	3	S8; S14; S23	Membrane lipid turnover; precursor pool for flavour volatiles
Glycine, serine and threonine metabolism	3	S11; S14; S21	Amino acid pool composition relevant to flavour and protein synthesis
PI3K-Akt signalling pathway	3	S5; S14; S18	Cell growth/survival signalling intersecting with muscle hypertrophy and lipid metabolism
Pentose phosphate pathway	3	S11; S20; S23	NADPH generation for antioxidant defence and fatty acid synthesis
Purine metabolism	3	S13; S14; S16	Nucleotide turnover; post-mortem energy charge; some flavour relevance
FoxO signalling pathway	2	S14; S22	Stress response and metabolic regulation pathway
Regulation of lipolysis in adipocytes	2	S11; S20	Direct regulator of intramuscular fat breakdown vs storage
Taurine and hypotaurine metabolism	2	S22; S23	Antioxidant amino-acid-derived metabolism

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Table 5. Methodological/quality assessment of included studies

Study	n/group	DEG threshold reported	qPCR/WB validation	True functional validation
S1	6 vs 6	|log2FC|≥1, *P* < 0.05	not stated	no
S11	10 vs 10	not specified	not stated	no authors explicitly state findings are pending future functional validation"
S16	6 vs 6	not specified	yes (qPCR + Western blot)	yes, *in vivo* mouse autophagy modulation (rapamycin/chloroquine)
S18	6/muscle	|log2FC|≥1, FDR < 0.05	yes (12 DEGs)	no qPCR confirms expression only
S22	6 vs 6	|log2FC|>1	yes (6 genes)	no qPCR confirms expression only
(20 others)	mostly n = 3–30/group	mostly not specified or *P* < 0.05 only	mixed	no in every remaining case

only 1 of the 27 included studies (S16; Han et al., 2025) reports any functional validation beyond confirming that a gene's expression changed

Figure 1. PRISMA 2020 flow diagram of the study selection process

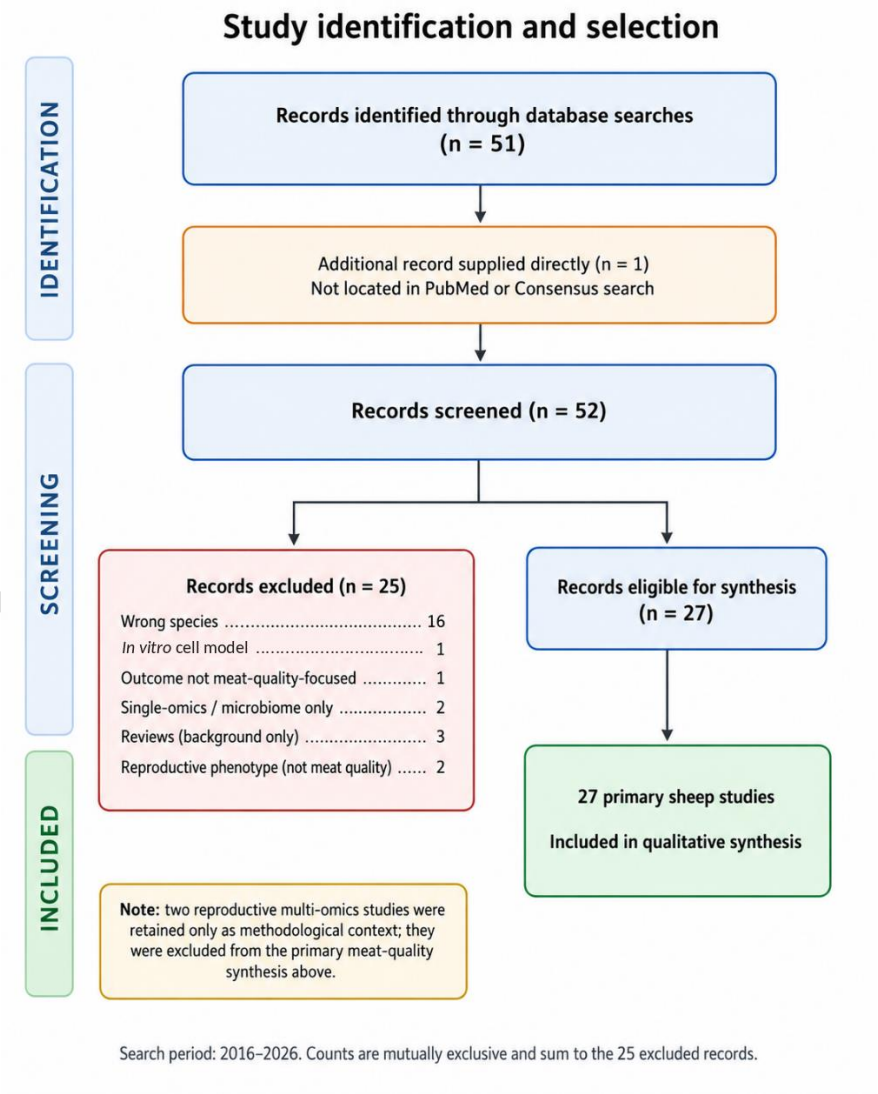


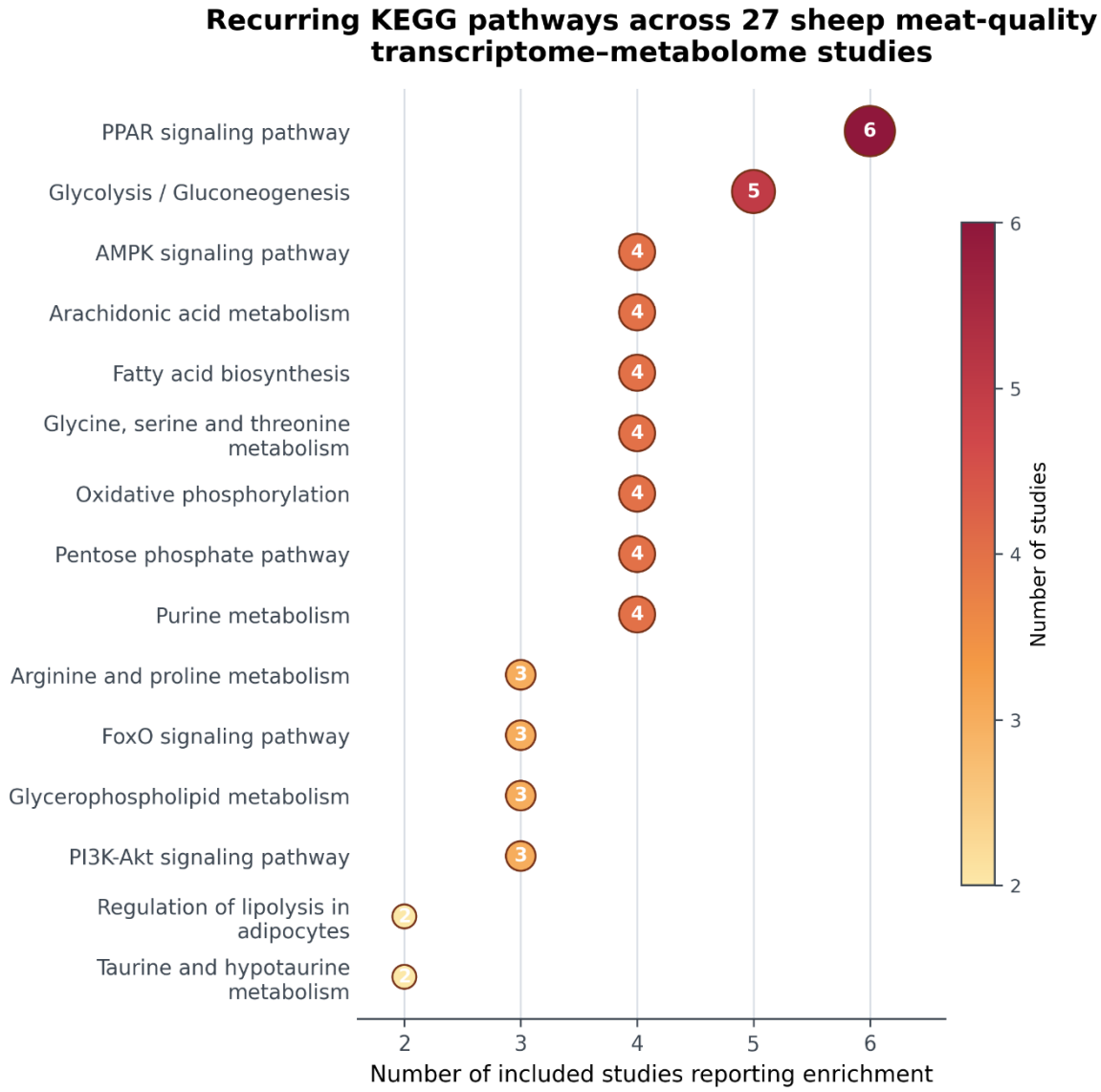
Figure 2. Overlap of named genes across the included studies

Named-gene overlap among the five studies with the most recoverable individual gene symbols



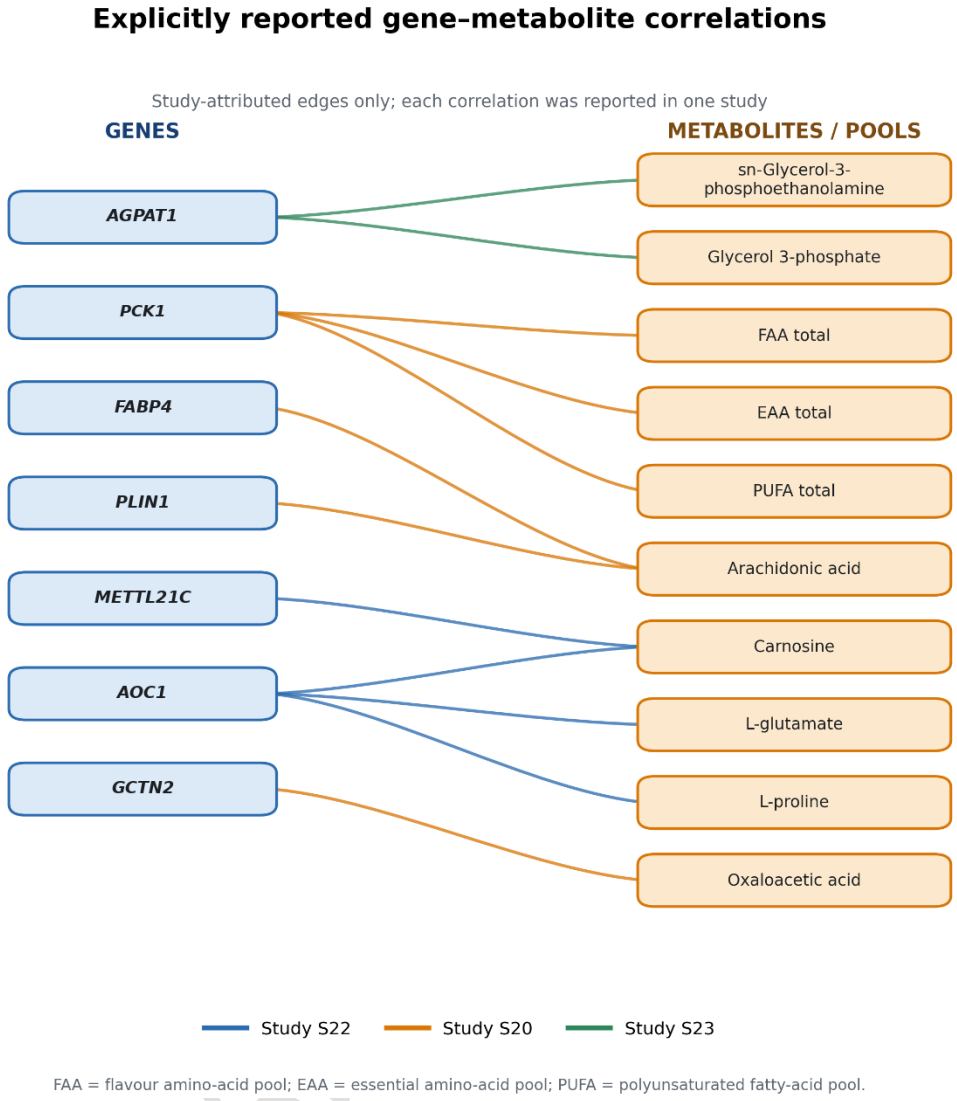
Only *PPARGC1A* is explicitly reported in more than one of these five studies. Ties in named-gene count were resolved by study ID.

Figure 3. KEGG pathways recurring across the included sheep meat-quality transcriptome–metabolome studies (counts as in Table 4)



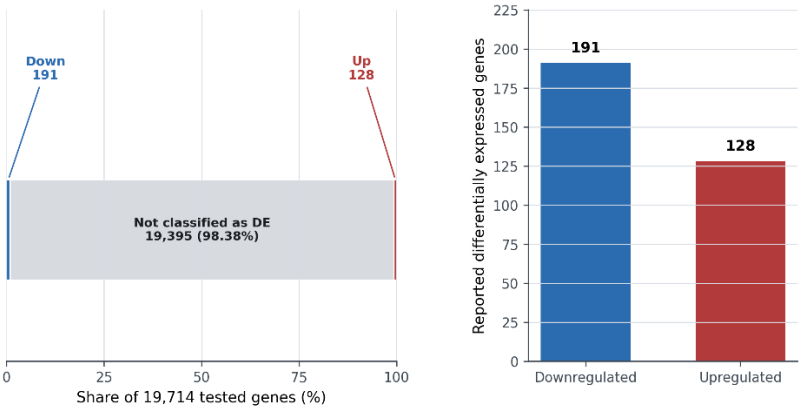
Bubble area and colour both encode the number of independent studies; study-level enrichment only (not pooled effect size).

Figure 4. Reported gene-metabolite correlations. Every edge derives from a single source study; no gene–metabolite pair was reported by more than one independent study.



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Figure 5. Differentially expressed genes reported by one representative included study: 191 down-regulated and 128 up-regulated transcripts of 19 714 tested. This panel illustrates a single study and is not a corpus-wide aggregate; original per-gene data were unavailable for the remaining studies, so no pooled DEG figure could be constructed.



[Original per-gene log2FC/P-value data were unavailable. This replacement reports the paper's published summary counts only; no simulated volcano plot is used.

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Figure 6. Heat map of named-gene reporting across the included studies

