

NUTRITIONAL EPIGENOMICS OF MICRONUTRIENTS IN RUMINANTS: FROM ONE-CARBON METABOLISM TO GENE REGULATION AND PHENOTYPIC PROGRAMMING-A REVIEW

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ABSTRACT

Nutritional epigenomics provides the mechanistic basis of how dietary nutrients can modulate gene expression without any permanent change in the genomic DNA sequence. In ruminants, this is particularly complicated and involves a unique sequence of events, in that nutrients are first transformed by rumen microbes, then metabolized by maternal and fetal tissues, and ultimately into molecular signals that impact chromatin configuration and gene expression. Micronutrients involved in one-carbon metabolism, antioxidant defense, mitochondrial function, and enzymatic activity may influence DNA methylation, histone modifications, chromatin structure, as well as expression of non-coding RNAs. These molecular reactions may bring about long-lasting alterations in growth, immunity, reproduction, metabolism, lactation and environmental response, particularly when nutritional exposure takes place in critical developmental stages. This review summarizes the current knowledge on folate, vitamin B₁₂, and choline-related methyl metabolism, and the associated vitamins B₂ and B₆, as well as zinc, selenium, iron, copper, manganese, iodine, and related micronutrients in the context of epigenetic regulation in the ruminant. In this context, there is a particular emphasis on one-carbon metabolism, methyl groups availability, oxidative stress, mitochondria, rumen microbial activity, and developmental programming. The effects of micronutrients are known to depend on dose, chemical type, bioavailability, physiological state, tissue, sex, genotype, and the underlying nutritional status. Nutritional epigenomics thus has great potential for precision feeding and improved health of the future animal, but translation will require additional causal evidence, standardized biomarkers, longitudinal phenotyping, and careful dissociation of adaptive programming from potentially deleterious molecular perturbation.

KEYWORDS: nutritional epigenomics; micronutrients; ruminants; one-carbon metabolism; DNA methylation; histone modification; gene regulation; precision nutrition

1. INTRODUCTION

Performance of ruminants has classically been explained by intake, digestion, absorption, and metabolism of nutrients. This perspective is still necessary but does not suffice to explain why exposures to nutrition in early life can alter productivity long after the initial insult has disappeared (Caton *et al.*, 2025). Nutritional epigenomics provides a mechanistic link between diet and long-lasting phenotype through investigation of the extent to which nutrients influence DNA methylation, histone modifications, chromatin accessibility, and regulatory RNA pathways. These processes influence whether genes are activated, suppressed or prepared for later stimulation. As a result, micronutrient nutrition could-not only impact immediate metabolism but also influence the life trajectory of an animal in terms of growth, reproduction, immune and disease response, and environmental adaptation (Crouse *et al.*, 2025).

The importance of this field is magnified in cattle, buffaloes, sheep and goats, as their nutritional environment is based on microbial fermentation. Rumen microorganisms make, modify, store or break down many vitamins and minerals before they are transported to host tissues. Absorbed nutrients are subsequently allocated among maintenance, pregnancy, lactation, immunity and tissue deposition, which establishes a physiological competition for limited metabolic resources (Badhan *et al.*, 2025). Thus, maternal diet can affect the fetal environment via alterations in circulating metabolites, placental function, endocrine signals, oxidative status and methyl-donor availability. As a result, nutrient-driven epigenetic regulation is a multilevel process linking feed composition, microbial ecology, host metabolism, reproductive biology and lifetime animal performance (Anas *et al.*, 2023).

Nutritional programming is of particular importance in the context of today's livestock production systems in which animals are subjected to potential high rates of growth, production, environmental stress, metabolic

pressure and variation in feed quality. Severe micronutrient deficiencies are known to impair classic biochemical pathways, yet marginal deficiencies may subtly alter transcriptional and epigenetic processes without manifesting as an overt clinical disorder. Overdosing may also affect redox balance, mineral interactions, and gene expression (Yazdanyar *et al.*, 2026). Hence the goal is not just to increase micronutrient consumption but rather to promote biologically relevant exposure at distinct developmental and physiological time points. Elucidating these relationships could facilitate precision nutrition to enhance resilience, efficiency and welfare while avoiding poorly targeted interventions based on oversimplified claims of permanent epigenetic enhancement (Ghaffari *et al.*, 2025).

2. Nutritional Epigenomics as a Regulatory Framework

Epigenetic mechanisms, which include molecular alterations during a cell's lifecycle, affect genomic activity without modifying the nucleotide sequence of DNA. DNA methylation predominantly occurs in cytosine-guanine dinucleotides and is linked to the context-specific activity of promoters, enhancers, transposable elements and gene bodies. Histone acetylation, methylation, phosphorylation and other posttranslational modifications of histone proteins modulate chromatin compaction or serve as docking sites for effector proteins (Millan-Zambrano *et al.*, 2022). Chromatin-remodelling complexes control the accessibility of genomic loci and transcription, RNA stability and translation are regulated by microRNAs, long non-coding RNAs and circular RNAs. These mechanisms are dynamic in their interactions, so that a micronutrient seldom exerts its action via one single avenue. Rather than acting directly, dietary cues influence the integrated metabolic, epigenetic and transcriptional networks (Evangelina *et al.*, 2025).

Nutrient-responsive epigenetic modifications include transient and reversible changes as well as stable developmental marks. Differentiated tissues continually adapt their epigenomes as a function of nutrient availability, inflammation, temperature and metabolic demand. In contrast, exposure to nutrition during gametogenesis, embryogenesis, placentation and fetal organogenesis could affect cell lineage commitment and set up regulatory states that are maintained through successive rounds of cell division (Halli *et al.*, 2025). Persistence of course need not imply irreversibility, and an observed methylation difference does not necessarily induce a phenotypic change. Some marks are a consequence of changed cell composition or metabolism. Classical nutritional epigenomics is therefore one of the exposure, metabolite availability, epigenetic modification, gene expression, cellular function and animal phenotype that composes a robust biological narrative (Smith *et al.*, 2025).

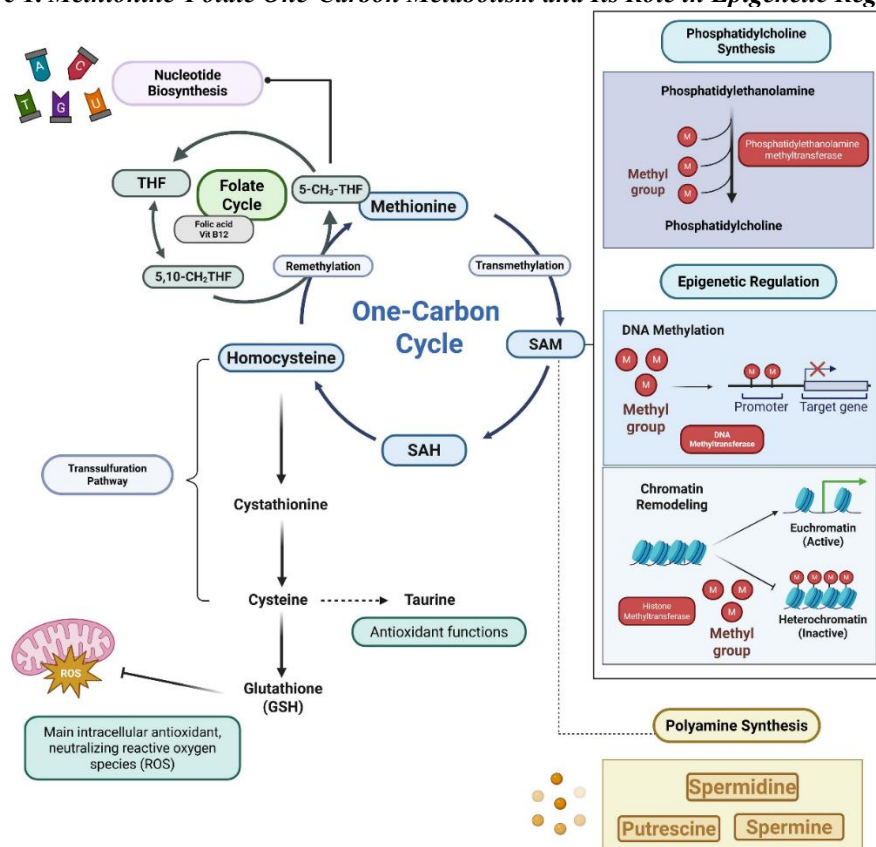
Micronutrient effects are determined by nutritional context, not just by supplementation. The response of animals that are deficient in a nutrient may be different from the response of animals maintained on adequate diets, and the same inclusion level may result in different tissue exposures because of ruminal degradation, antagonistic minerals, forage composition or physiological demand. Responses are also influenced by genetic polymorphisms in transporters and metabolic enzymes. Age, sex, parity, breed, production stage and health status are also important epigenetic sensitizers (Weiss and Hansen, 2024). Thus, nutritional epigenomics should be understood as a nutrient-by-genotype-by-environment interaction. This view does not favor universal supplementation recommendations but rather treatments tailored to basal status, biological timing, chemical form and measurable functional outcomes (McKnight *et al.*, 2025).

3. One-Carbon Metabolism: The Metabolic Core of Epigenetic Regulation

The one-carbon metabolism network links the folate cycle, methionine cycle, and transsulfuration pathway. This network provides one-carbon units for nucleotide synthesis, amino-acid metabolism, redox regulation, and methylation. Methionine is the precursor of S-adenosylmethionine (SAM), the primary methyl donor for DNA, histone, RNA, protein and lipid methylation. SAM becomes S-adenosylhomocysteine (SAH) after donating its methyl group, and SAH is subsequently hydrolyzed to homocysteine. The SAM:SAH ratio is a key determinant of cellular methylation potential, as SAH is a potent inhibitor of methyltransferases. Homocysteine can be remethylated to methionine via folate and vitamin B₁₂-dependent enzymes or enter the vitamin B₆-dependent transsulfuration pathway to generate cysteine and serve as a precursor for glutathione synthesis (Tassinari *et al.*, 2024; Bernasocchi and Mostoslavsky, 2024; Flori *et al.*, 2025).

One-carbon units are donated to the folate cycle mainly by serine, glycine, and similar metabolism. Tetrahydrofolate derivatives transport these units and channel them to nucleotide synthesis or to regeneration of methionine. The methyl group from methylated folate is transferred to homocysteine by the B₁₂-dependent methionine synthase to regenerate methionine and tetrahydrofolate, and riboflavin-derived cofactors and vitamin B₆ aid in related reactions (Mucha *et al.*, 2024; Dang *et al.*, 2024). Transsulfuration pathway also connects methyl metabolism to antioxidant defence via glutathione synthesis. One-carbon metabolism, therefore, is dependent on the balance of competing processes including methylation, nucleotide synthesis, protein and phospholipid synthesis, and redox maintenance. Fluctuations in oxidative stress may drive metabolism towards glutathione synthesis, thereby altering methyl-donor availability and epigenetic regulation (Ren *et al.*, 2024).

Figure 1. Methionine-Folate One-Carbon Metabolism and Its Role in Epigenetic Regulation



Source- Costa *et al.*, 2025

One-carbon metabolism is also highly coupled with mitochondrial function which produces formate and modulates amino acid metabolism, energy status and redox homeostasis. Mitochondrial dysfunction may affect the supply of SAM and other metabolites, including acetyl-CoA, α -ketogutarate, succinate, fumarate, and NAD⁺, which have an impact on enzymes that modify chromatin (Zhang *et al.*, 2025). Increased methyl-donor availability is not necessarily translated into DNA global hypermethylation as cells control nutrient transport, enzyme activity, cofactors, compartmentation, and substrate utilization. Methyltransferases are selective at genomic sites, supplementation might enhance methylation at some loci, reduce it at others, or not change much. Hence, the micronutrient-epigenetic interaction should be studied with methylation analysis alongside gene expression, metabolic profiling and phenotypic measures while taking account of tissue type, developmental stage, and physiological stress (Oliveira *et al.*, 2025; Leesang *et al.*, 2024; Ivanova and Kelsey, 2025).

4. Micronutrients Influencing the Ruminant Epigenome

4.1 Folate

Folate provides activated one-carbon units for the synthesis of nucleotides and remethylation of homocysteine. Folate is required for DNA replication, cell proliferation, and maintenance of methyl-donor pools, and is especially critical during embryogenesis, placentation, and rapid fetal tissue growth. While rumen microbes in adult ruminants produce folates, the amounts produced may not always meet the requirements of high producing or fast growing animals (McFadden *et al.*, 2020). In addition, dietary folate can be degraded in the rumen, leading to inconsistent post-ruminal availability. Protected folate sources may enhance delivery, but their biological value is determined not only by dietary concentration but also by stability, absorption, and tissue utilization (Girard and Duplessis, 2023).

Folate deficiency can perturb nucleotide synthesis, promote DNA damage and alter methylation patterns, while too much or unbalanced supply can divert one-carbon units without producing helpful programming. Maternal folate status may also affect genes involved in placental transport, hepatic metabolism, myogenesis, adipogenesis, and immune development (Mellor *et al.*, 2025). However, responses vary in different stages of gestation, as early embryos are subjected to global epigenetic reprogramming while later fetal tissues represent more highly specialized chromatin models. Folate supplementation should possibly take into account the time of cell-lineage specification and organ development. The most informative biomarkers include circulating and tissue folate species, homocysteine, methionine cycle metabolites, methylation capacity and activity of enzymes involved in folate transport and metabolism (Castillo *et al.*, 2025).

4.2 Vitamin B₁₂ and Cobalt

Vitamin B₁₂ is necessary for methionine synthase and methylmalonyl-coenzyme A mutase. The first enzyme couples cobalamin directly with methionine resynthesis and methyl metabolism, whereas the second enzyme serves propionate metabolism and entry of carbon into energy metabolism. In ruminants, the vitamin B₁₂ is produced by rumen bacteria in the presence of adequate supply of cobalt. Consequently, cobalt deficiency might affect methyl metabolism as well as energy metabolism, leading to effects that are unlikely to be caused by epigenetic modification alone. The amount and species of corrinoids produced are influenced by the microbial composition, the dietary form of cobalt, passage through the rumen, and by antagonistic factors, and not all exhibit the same level of biological activity (Arce-Cordero *et al.*, 2025).

Maternal vitamin B₁₂ status can affect the turnover and distribution of one-carbon metabolites in maternal circulation, fetal fluids, placenta and the developing organs. Transport or fetal expression of one-carbon enzymes may adapt in response to nutrient restriction, such that circulating concentration is not always an accurate indicator of cellular sufficiency (Safain *et al.*, 2025). Low cobalamin can result in a functional folate trap, impair methionine regeneration and change the S-adenosylmethionine to S-adenosylhomocysteine ratio. This can potentially lead to changes in hepatic gene expression, impaired growth and altered metabolic maturation. Different supplementation strategies must consider the source of cobalt, microbial synthesis, absorbable vitamin production and the specific requirements of gestation, growth and lactation (Syring *et al.*, 2024).

4.3 Vitamins B₂ and B₆

Riboflavin and vitamin B₆ are facilitators of one-carbon metabolism. Flavin adenine dinucleotide (FAD), a metabolite of riboflavin, acts as a cofactor for enzymes, which are important for the metabolism of folate and the methionine-cycle. Pyridoxal phosphate, an active metabolite of vitamin B₆, also facilitates serine-glycine metabolism and remethylation of homocysteine via transsulfuration (Pentieva *et al.*, 2005). Thus, their availability may determine whether one-carbon units fuel methylation, nucleotide synthesis or antioxidant defence. The synthesis of these vitamins is performed by rumen microorganisms, although high production, fermentation alteration, disease or antagonistic feedstuffs can affect their bioavailability. Their epigenetic significance will probably be realized as network effects as opposed to direct binding to chromatin (Brisson *et al.*, 2022).

4.4 Choline, Betaine and Methionine-Associated Nutrition

Choline is not normally considered a vitamin in all nutritionalities yet its essential for making sense of micronutrient-fueled one-carbon metabolism. It is involved in phosphatidylcholine production, lipoprotein formation, cell membrane, and generation of methyl groups subsequent to its oxidation to betaine. In addition to the folate and vitamin B₁₂ pathways, betaine-mediated remethylation of homocysteine is an alternative remethylation pathway, which offers the system some metabolic leeway during limitation of the folate-dependent pathway. As free choline is largely degraded in the rumen, rumen-protected products are often evaluated close to the transition period and during gestation. Their impacts may include hepatic lipid export, inflammation regulation, placental function, colostrum biology and progeny growth besides alterations in methyl-donor availability (Arshad and Santos, 2024).

Methionine is an essential amino acid and the precursor of S-adenosylmethionine but it is not only a methyl donor for protein synthesis; it is also implicated in the formation of polyamines, in antioxidant defenses and in metabolic signalling (Espe *et al.*, 2023). Rumen-protected methionine may influence mammary, liver, placental, and immune transcription through amino-acid sensing and epigenetic mechanisms. Hence, these choline, betaine, methionine, folate and vitamin B₁₂ should be viewed as a single, integrated metabolic entity. The addition of one factor could expose the limitations of another or divert flow toward other competing functions. This is why such balanced interventions guided by metabolite profiling represent a more trustworthy strategy to dissect epigenetic responses relative to single-nutrient interventions that have only been evaluated in terms of production traits (Osorio *et al.*, 2016).

4.5 Zinc

Zinc is a structural, catalytic, and signalling ion that plays a role in epigenetic regulation. Many transcription factors have zinc-finger domains that bind DNA and recruit chromatin-regulatory complexes. Zinc is also a cofactor for enzymes involved in DNA synthesis, repair, antioxidant protection, and cellular differentiation. Zinc deficiency can increase oxidative damage and inflammatory signalling, which can indirectly alter chromatin states and expression of microRNAs (Kamaliyan and Clarke, 2024). Zinc availability may affect myogenesis, immunity, intestinal maturation, and reproductive development in growing ruminants. But excess zinc can compete with copper absorption and disrupt mineral homeostasis. Organic and inorganic zinc sources may have different ruminal fates, absorption, and tissue delivery, adding another layer of complexity to the interpretation of epigenetic results (Kesler and Abuelo, 2024).

Zinc-dependent pathways often regulate the same genes involved in barrier function, cytokine expression, antioxidant protection, and cell growth. Zinc status can modulate transcriptional circuits that influence health and production in the mammary gland, liver, intestine, and immune cells. Epigenetic mediation is feasible for these alterations when they consist of methylation, histone modifications or regulatory RNAs, but direct causality is still not confirmed in ruminants (Sindhu *et al.*, 2024). Additional work should separate zinc-responsive transcription from stable epigenetic programming by assaying chromatin marks with gene

expression. Dose-response studies are particularly necessary to verify as deficiency replenishment, optimal supply and pharmacological exposure are biologically distinct states, with possibly contradictory outcomes (Li *et al.*, 2025).

4.6 Selenium

Selenium is a constituent of selenoproteins, involved in the regulation of antioxidant defence, thyroid hormone metabolism, protein quality control and redox signalling. Selenium may affect redox-sensitive TFs and the activity of epigenetic enzyme by modulating reactive oxygen species. It is possible that oxidative stress alters the expression of DNA methyltransferase, histone modification and microRNA patterns, thereby indirectly allowing Se to affect gene regulation (Shahidi *et al.*, 2025). Maternal selenium nutrition has also been linked to alterations in placental function, fetal growth, immune development and antioxidant capacity. Outcomes vary by baseline selenium status, chemical form, dose, and the competing processes of remedying deficiency and moving toward toxic exposure (Cruickshank *et al.*, 2025).

Organic selenium compounds and inorganic salts have different absorption, retention and metabolic incorporation. Such differences may result in different molecular effects even at similar levels of selenium intake. Selenium supplementation can influence the expression of genes related to glutathione metabolism, inflammatory responses, muscle growth and endocrine regulation, but not all transcriptional responses are epigenetic programming (McDermott *et al.*, 2024). Longitudinal studies are needed to determine if prenatal selenium exposure results in long-term methylation or chromatin changes once postnatal environments are standardized. Selenium is a trace element with a relatively narrow range between adequacy and toxicity, and thus epigenomic findings should never be used to justify indiscriminate high-level supplementation (Farang *et al.*, 2025).

4.7 Iron, Copper and Manganese

Iron is involved in oxygen transport, mitochondrial respiration, and many dioxygenase reactions. Iron and α -ketoglutarate are also required for the activity of several enzymes that catalyze DNA and histone demethylation reactions, linking iron status with mitochondrial metabolism and chromatin regulation. Both iron deficiency and iron overload can disrupt this balance. Iron deficiency can compromise energy metabolism and enzyme activity, while iron overload promotes oxidative damage (Ben Zichri-David *et al.*, 2025). In ruminants, fetal iron supply and neonatal iron status may affect the development of the nervous, immune and metabolic systems. But forage contamination, soil ingestion and interactions with other minerals are all potential sources of iron exposure, making assessment of iron intake difficult and emphasizing the need to measure bioavailable iron rather than total dietary iron (Yu and Li, 2024).

Copper is a cofactor for cytochrome oxidase, superoxide dismutase, elastin formation and cross-linking, pigmentation and iron metabolism. Via these pathways, copper regulates mitochondrial function, oxidative homeostasis, and cell signaling, which are potential targets of epigenetic modifications. Deficiency of copper during pregnancy may alter placentation, fetal growth, cardiovascular development and immunologic competence (Ran *et al.*, 2026). Copper overload can also induce oxidative stress and consequent cell death in the liver, especially in predisposed sheep. Molybdenum, sulfur, and iron interfere with copper absorption by antagonism. Therefore, epigenomic studies have to measure the ratios of minerals and metabolic markers rather than considering the concentration of dietary copper separated from the whole feeding environment (Anjana *et al.*, 2025).

Manganese is involved in mitochondrial antioxidant defence, carbohydrate metabolism, bone development and reproduction (Studer *et al.*, 2022). Manganese-dependent enzymes can also define metabolic conditions that affect the availability of chromatin substrates and transcriptional signalling. Deficiency during development in an organism may impair bone development, reproduction, and cellular oxidative status and environmental exposure too high may cause neurotoxicity. Direct evidence linking manganese intake to stable epigenetic programming in ruminants is still scarce; however, mechanistic plausibility warrants more investigations. Future studies should investigate mitochondrial function, oxidative biomarkers, DNA methylation and gene expression in parallel. This integration would determine whether manganese influences epigenetic regulation per se or whether the molecular changes observed in manganese deficiency, growth retardation, oxidative stress and tissue injury are derivative (Aschner *et al.*, 2025).

4.8 Iodine and Thyroid-Dependent Programming

Iodine affects gene expression mostly through thyroid hormones, which interact with nuclear receptors and recruit transcriptional coactivators, corepressors and chromatin-remodelling proteins (Bernal, 2007). Maternal iodine deficiency can decrease fetal thyroid hormone availability during the formation of neural, muscle and metabolic tissues. Thyroid signalling also regulates DNA methylation and histone modifications at developmentally regulated genes. High iodine levels can inhibit thyroid function or cause its deregulation, so the optimal level is required, not the maximum one. In grazing systems, forage iodine, soil parameters, goitrogenic plants and dietary antagonists affect the actual status. Thus, epigenetic studies should integrate iodine exposure with maternal and fetal thyroid measurements and tissue-specific chromatin measurements (Moreno-Reyes *et al.*, 2025).

4.9 Micronutrient Interactions

Micronutrients act as a network rather than individual dietary components. Vitamin B₁₂ is needed for the proper regeneration of methionine from homocysteine, while vitamin B₆ helps to route homocysteine down the

transsulfuration pathway, and riboflavin is a cofactor for enzymes in the folate cycle. Zinc, copper, manganese, iron, and selenium are involved in the regulation of oxidative balance, but can also compete for absorption or modify each other's metabolism (Byrne and Murphy, 2022). Thyroid activity, which is dependent on iodine, also plays a role in energy expenditure and the expression of developmental genes. Thus, a deficiency in one nutrient may modify the observed response to another. Nutritional epigenomic studies should use factorial or mixture designs, report the full diet, and measure relevant antagonists to avoid drawing conclusions from isolated supplement labels (Castillo *et al.*, 2025).

5. Molecular Pathways Linking Micronutrients with Gene Regulation

5.1 DNA Methylation

DNA methylation is the most studied mechanism in nutritional programming. DNA methyltransferases catalyze the transfer of methyl groups from S-adenosylmethionine to cytosine, while active demethylation is mediated by oxidation and repair pathways that rely on metabolic cofactors. Thus, micronutrients can affect both methyl-group supply and demethylating activity. Methylation in a promoter region can inhibit transcription, but this is not always the case; methylation in enhancers, gene bodies or repetitive elements can have different implications (Greenberg and Bourc'his, 2019). In addition, bulk tissue analysis may confound real methylation changes with changes in cell composition. High-resolution cell-aware methods are needed to identify which regions of the genome respond and whether those responses regulate function (Geisenberger *et al.*, 2025).

5.2 Histone Modifications and Chromatin Accessibility

Modifications of histone create a dynamic interface between cellular metabolism and transcription. Histone acetylation relies on acetyl-coenzyme A and is erased by a class of deacetylases that respond to metabolic and redox states (Sabari *et al.*, 2017). Histone methylation consumes S-adenosylmethionine, whilst several demethylases are dependent on iron, α -ketoglutarate and oxygen (Reid *et al.*, 2017). Changes in mitochondrial function induced by micronutrients are thus capable of affecting chromatin even in the absence of significant change in DNA methylation status. Chromatin-remodelling complexes then move nucleosomes and facilitate transcription-factor binding. These processes, particularly in ruminant tissues, may govern metabolic adaptation to the periparturient period, immune responses and mammary growth, but are less comprehensively defined relative to DNA methylation and transcript abundance (Charidemou and Kirmizis, 2024).

5.3 Non-Coding RNAs

MicroRNAs control the pools of mRNAs by inducing their degradation or translation repression. Long non-coding RNAs can recruit chromatin-modifying complexes, modulate nuclear architecture and transcription, and circular RNAs can act as microRNA sponges or bind to proteins (Bartel, 2018; Statello *et al.*, 2021). The expression of non-coding RNA can be modified by micronutrients via oxidative, endocrine and metabolic signalling. In fact, such responses have been detected in inflammation, muscle development, adipogenesis, mammary and placental function-related pathways. Since each microRNA has the potential to target a large number of genes, subtle dietary alterations could lead to widespread network consequences. Validation should entail target validation and functional assay, not just data mining based on predicted associations from sequence databases (Khandelwal *et al.*, 2024).

5.4 Transcription Factors and Nutrient-Sensing Pathways

Micronutrients modulate transcription factors and nutrient-sensing pathways that converge on cellular energy, amino acid, oxidative stress and endocrine signals. Nuclear receptors, antioxidant response factor, hypoxia sensitive molecules, inflammatory mediators and growth-related signalling pathways can also attract epigenetic modifiers to specific genomic locations. This targeting is responsible for the fact that a general perturbation in the availability of methyl-donors leads to locus-specific rather than uniform epigenetic alterations. Methionine-related signalling may regulate translation and selenium, zinc, copper and manganese regulate redox-sensitive signal transduction pathways. Thyroid hormones constitute a further mechanism by which iodine status may influence developmental transcription. Epigenetic and transcriptional responses should thus be investigated as integrated outcomes and not as competing interpretations (Jomova *et al.*, 2025; Hernandez *et al.*, 2023).

5.5 Epigenetic Crosstalk and Cellular Memory

Epigenetic processes hardly ever work in isolation. DNA methylation can reinforce repressive chromatin, and histone modifications can attract DNA methyltransferases or demethylases and ncRNAs can guide regulatory complexes to particular loci. Nutritional exposure may first exert an effect on transcription-factor activity followed by chromatin changes that lock in or alter subsequent responsiveness. This cellular memory might enable adaptation to predictable patterns of nutrient or environmental availability, but it might also limit physiological plasticity. To demonstrate memory, one needs to show molecular and phenotypic effects lasting beyond the period of nutritional exposure. In the absence of such follow-on transient nutrient-responsive transcription should not be equated with persistent epigenetic reprogramming (Tomljanovic *et al.*, 2025).

5.6 Micronutrient-Responsive Genes in Epigenetic Regulation and Phenotypic Programming

Micronutrient-responsive genes connect one-carbon metabolism with epigenetic regulation and economically important traits in ruminants. MTHFR, MTR, MAT1A, MAT2A, AHCY and BHMT regulate folate cycling, methionine regeneration and S-adenosylmethionine availability, thereby determining cellular methylation potential. DNMT1, DNMT3A and DNMT3B maintain or establish DNA methylation, whereas TET1-3 promote active demethylation. Histone-regulating genes, including EP300, CREBBP, HDACs, SIRT1, SIRT3 and EZH2,

modify chromatin accessibility and transcriptional activity. Selenium-, zinc-, copper- and manganese-responsive genes such as GPX1, GPX4, SELENOP, MT1A, SOD1, SOD2 and NFE2L2 coordinate antioxidant protection and cellular stress responses. DIO1-3, THRA and THRB connect iodine and selenium with thyroid-dependent growth and metabolism. Downstream targets, including IGF2-H19, MYOD1, MYOG, MSTN, PPARG, PPARGC1A, TLR4, STAT5A, FASN and SCD, influence fetal growth, muscle differentiation, adipogenesis, immunity, mammary development and milk-fat synthesis, translating maternal micronutrient exposure into persistent productive and adaptive phenotypes (Anas *et al.*, 2023).

6. The Rumen Microbiome-Epigenome Axis

The rumen microbiome mediates many host exposures to compounds with potential epigenetic effects. Microbes synthesize B vitamins, convert cobalt to corrinoids, degrade dietary choline, and metabolize substrates that contain sulfur and nitrogen. Fermentation results in volatile fatty acid production that serves as an energy source to host metabolism and modulates endocrine and immune signaling. Therefore, dietary interventions can indirectly alter the host epigenome by remodeling the gut microbial populations and their metabolite profiles. The rumen is not to be regarded only as a source of nutrient loss or gain: it is a biological processor that defines the chemical forms and the delivery schedules of regulatory metabolites to the intestine, liver, placenta and peripheral tissues (Ye *et al.*, 2025; Nath, 2026).

Butyrate is especially relevant since it may regulate histone acetylation and cell differentiation. In the rumen, most butyrate is utilized by the epithelium, but alterations in its production may affect rumen development, epithelial gene expression, and systemic metabolism. Propionate supports hepatic gluconeogenesis and metabolism dependent on vitamin B₁₂, connecting fermentation against carbon utilization and methyl-related metabolism. Microbial folates and other B vitamins contribute to the host folate and B vitamin supply, but production is dependent on diet, ruminal pH, and community composition. As a result, epigenome-targeted dietary interventions could have combined effects on direct nutrient supply, microbial modifications, and fermentation-dependent signaling, complicating the mechanistic dissociation in experimental models (Alhasyani *et al.*, 2025).

The microbiome itself can also be programmed by maternal nutrition, birth environment, colostrum, milk feeding and early solid-feed exposure. Early microbial succession may affect epithelial maturation, immune education, and metabolic signalling during critical developmental windows. Host epigenetic regulation can, in turn, influence barrier function, secretions and immune responses that modulate microbial habitats. This is not a one-way road but a microbiome-epigenome axis. Multi-omics studies using microbial metagenomes, fermentation metabolites, host methylomes, chromatin states and transcriptomes will be required to identify causal intermediates and to differentiate microbial mediation from parallel responses to the same diet (Diddeniya *et al.*, 2024).

7. Critical Windows of Developmental Programming

7.1 Gametes and Early Embryos

Gametes and preimplantation embryos experience massive epigenetic reprogramming, and consequently conception and early pregnancy are very vulnerable to maternal and paternal nutrition. Micronutrient status can affect oocyte competence, sperm quality, fertilization, embryonic metabolism and the generation of regulatory marks. Small changes in one-carbon availability can affect this process during a time of rapid DNA replication and methylation reprogramming. But there is a selection bias that can arise from the embryo survival effects, where only developmentally competent conceptuses are available for later analysis. Thus, studies should aim to differentiate between effects on survival and true programming among surviving offspring. While maternal and paternal exposures are both relevant, the role of paternal micronutrient programming in ruminants is still under-investigated (Sinclair, 2025).

7.2 Placentation

The placenta, mediating nutrient transfer, endocrine signaling, oxygen exchange, and immune tolerance between maternal and fetal circulation. Its epigenome responds to the maternal supply of nutrients and may modify transport capacity in conditions of supply restriction or imbalance. Folate, vitamin B₁₂, choline-derived metabolites, selenium, zinc, iron, copper and iodine have the potential to modulate placental cell proliferation, vascularization, antioxidant defence system and hormone production. Placental adaptation may serve to maintain fetal growth in the short term; however, compensation can result in redistribution of blood flow among organs, leading to altered metabolic function later in life. Placental tissues, therefore, should be examined in combination with fetal tissues, maternal blood and fetal fluids to accurately reconstruct transplacental nutrient passage and regulatory outcomes (Casarotto *et al.*, 2026; Caton *et al.*, 2025).

7.3 Fetal Organogenesis

Organogenesis is the result of high levels of cell proliferation, differentiation and acquisition of tissue-specific chromatin states. Exposure to nutrition during this stage might affect the number of and/or function of muscle fibres, adipocytes, pancreatic cells, hepatocytes, immune cells and ovarian follicles. A variation in cell number cannot always be rescued by post-natal feeding, which accounts for the persistence of prenatal programming. The stage of development at which the pertinent organ and cell line are forming determines the impact of a micronutrient. Hence, gestation should not be conceptualised as a single exposure period. Whole-pregnancy interventions, in contrast, may mask narrower windows of greatest benefit or harm (Johnson *et al.*, 2025; Penagaricano *et al.*, 2014).

7.4 Late Gestation and the Perinatal Period

High rates of fetal growth, mineral accretion, immune maturation and metabolic demand are characteristic of late gestation. Maternal appetite may decrease due to the pressure exerted by the growing fetus on the abdominal cavity, especially under heat stress or poor forage conditions. This can influence colostrum composition, neonatal antioxidant status, metabolic preparedness and passive immune transfer. However, molecular effects associated with prenatal programming may be further augmented or overridden by colostrum intake, milk composition, neonatal supplementation and disease exposure. Cross-fostering, standardized colostrum management and careful documentation of early-life treatments are required to distinguish between prenatal effects and postnatal nutritional continuation. These controls are required to assess persistent offspring phenotypes (Cattaneo *et al.*, 2022; Upadhyay *et al.*, 2026).

7.5 Postnatal Growth, Puberty and Lactation

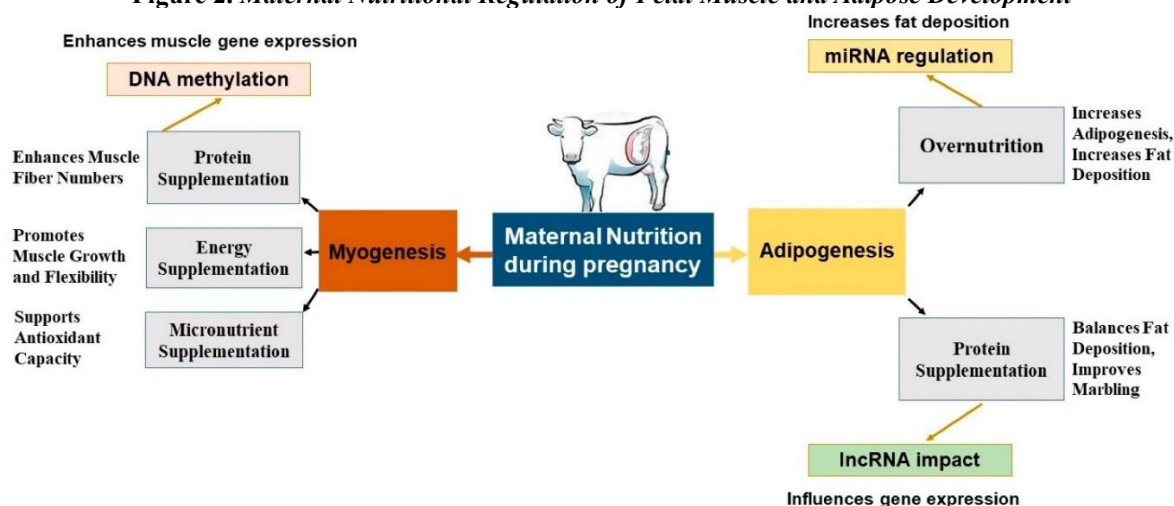
Epigenetic plasticity persists after birth, albeit to a lesser extent and with different biological consequences than embryonic reprogramming. Weaning, diet transition, puberty, pregnancy and first lactation are all metabolic stressors that can trigger changes in gene expression. Early-life micronutrient nutrition may affect rumen maturation, immune function, bone development and reproductive maturation. Subsequent exposures may either reinforce, compensate for or uncover prenatal programming. A phenotype programmed during prenatal life may not be apparent until the animal is challenged with heat, infection, undernutrition or lactation. Thus, longitudinal studies that extend beyond growth measurements are required to establish whether epigenetic differences are associated with improved resilience, reproductive performance, feed efficiency, milk yield or productive longevity (Halli *et al.*, 2025; Harati *et al.*, 2024).

8. Phenotypic Consequences of Micronutrient-Driven Programming

8.1 Growth, Muscle and Carcass Development

Skeletal muscle development involves myoblast proliferation, differentiation, fibre formation, vascularization and metabolic maturation. These processes are regulated by transcriptional and epigenetic networks that integrate signals from methyl donors, zinc, selenium, iron and thyroid hormones. Maternal micronutrient status may affect the number of muscle fibres formed in the fetus, postnatal hypertrophic potential, mitochondrial function and oxidative stability. Moreover, alterations in adipogenic programming may also influence fat deposition, marbling and metabolic efficiency. However, increased birth weight is not necessarily associated with improved lifetime performance; thus, studies need to incorporate body composition, muscle histology, feed efficiency and carcass traits, along with relevant molecular markers, to assess whether the observed programming is biologically and economically beneficial (Gundersen and Anas, 2025).

Figure 2. Maternal Nutritional Regulation of Fetal Muscle and Adipose Development



Source- Shokrollahi *et al.*, 2025

8.2 Immunity and Disease Resilience

The coordinated development of innate and adaptive cell populations, the induction of tolerance and the control of inflammation are all required for immune development. Zinc, selenium, copper, iron, folate, and vitamins B₆ and B₁₂ are involved in immune cell proliferation, antioxidant defence and cytokine regulation. DNA methylation, histone states and microRNAs responsive to nutrients may modulate immune cell responsiveness to infection or vaccination. Programmed nutrition should enhance robust defence without inducing chronic inflammation or excessive tissue damage. Thus, functional challenges, vaccine responses, pathogen resistance and recovery measurements are more informative than resting gene expression. Immune phenotypes should also be considered in the context of passive transfer and early microbial colonization (Cangiano *et al.*, 2024; Olszowski and Chlubek, 2026).

8.3 Reproduction

Reproductive characteristics might also be affected by exposure to micronutrient at diverse stages of cellular development through several generations. Maternal diet influences fetal ovarian reserve, fetal testis development, hypothalamic-pituitary signaling and sexual maturation. Zinc and selenium, copper, iodine, folate and vitamin B₁₂ play essential roles in germ-cell development, endocrine regulation and defense against oxidative stress. Reproductive tissue-specific epigenetic changes may affect puberty, gamete quality, conception and embryonic survival. However, growth rate, body condition, disease and husbandry all influence reproduction, and these effects are hard to separate. Longitudinal studies should integrate molecular read-outs with age at puberty, semen quality, ovarian function, conception rate, pregnancy maintenance and calving outcomes (Cushman *et al.*, 2024).

8.4 Mammary Development and Lactation

The mammary gland is subjected to considerable developmental and epigenetic reprogramming at puberty, pregnancy and lactation. Maternal and early-life micronutrients supply could have an effect on mammary epithelial proliferation, vascular development, immune protection and subsequent secretory potential. Genes involved in lipid synthesis, protein synthesis, cell differentiation and inflammation may be regulated by methyl-donor metabolism, antioxidant micronutrients and thyroid signaling. In addition, in mature cows, the combined effects of rumen-protected methionine and choline supplementation on hepatic-mammary nutrient partitioning and transcription were evaluated. There is great potential for persistent effects of prenatal exposures on adult milk production, but the evidence is inconclusive due to the lack of studies that report multiple lactations (Laporta and Guenther, 2025).

8.5 Metabolic Efficiency and Environmental Adaptation

Micronutrient-dependent programming could affect hepatic gluconeogenesis, fatty acid oxidation, insulin action, mitochondrial function and nutrient partitioning. This is what modulates the biological responses of the animal to feed restriction, transition, lactation and thermal load. Epigenetic modification is adaptive if it readies offspring for an environment similar to that of the dam. A mismatch between prenatal prediction and postnatal environment may exacerbate metabolic susceptibility. Faster growth or production should not be taken too literally as signs of improved efficiency, since they may be associated with increased maintenance needs or reduced robustness. Feed intake, digestibility, methane emissions, body reserves, health and reproductive success should all be evaluated in unison when assessing programmed efficiency (Diniz *et al.*, 2023).

9. Experimental and Interpretative Challenges

Nutritional epigenomics is a difficult area in terms of design. Several gestational interventions, tissue sampling, and long-term follow-up are expensive, thus many ruminant studies are conducted on small cohorts. Small sample size reduces power and increases chance of false discovery in genome-wide studies. Results may be confounded by age of the mother, parity, breed, body condition, number of fetuses, sex of the offspring and genetics of the sire. Animals in the same pen also have common environment and microbial exposures, thus for certain treatments the pen rather than the individual is the de facto experimental unit. Solid studies need to have pre-registered hypothesis, proper replication, balanced design, independent validation and transparent reporting of complete dietary composition (Percie du Sert *et al.*, 2020).

Tissue selection is also critical, as epigenetic regulation is largely cell and tissue-specific. Blood can be repeatedly sampled, but may not represent liver, muscle, placenta, mammary gland, or reproductive tissues. Differences in leukocyte proportions may manifest as methylation differences, even if methylation is constant within each cell type. Single-cell and spatial technologies can overcome cellular heterogeneity, but are still hampered by cost and lack of ruminant genome annotation. Additionally, sampling time needs to be relevant to the biological question, as some nutrient-responsive marks can arise and disappear quickly. Repeated sampling along with terminal tissue collection provides a better understanding of the progression and persistence over time (Lyons *et al.*, 2024; Wang and Ibeagha-Awemu, 2021).

Multi-omics analyses have the potential to link nutritional exposure to mechanisms more directly than single endpoint measures. Metabolomics can measure one-carbon intermediates and redox metabolites, epigenomics can detect regulatory alterations, transcriptomics can detect functional gene expression and proteomics can assess whether the transcriptional alterations are translated into proteins. Microbiome and phenotypic information guide the full path from diet to performance. Integration should be driven by biological hypotheses, not by the amount of data being integrated. Causal mediation analyses, genetic variation, and temporal ordering and controlled perturbations can be used to distinguish regulatory mechanisms from correlated biomarkers. Functional validation remains required for an epigenetic signature to be regarded as a nutritional target (Kim and Kim, 2021; Wadood *et al.*, 2025).

10. Precision Nutrition and Responsible Application

Precision nutritional epigenomics is a strategy to optimize micronutrient supply according to the animal's genotype, physiological state, basal status, production environment and developmental stage (Naeem and Fatima, 2025). Practical tools include a combination of forage and feed analysis, blood biomarkers, rumen indicators, genomic information and longitudinal performance records. Rather than applying uniform supplementation, producers could target those animals or groups most likely to respond to protected vitamins, organic trace minerals or modified feeding schedules. These systems must remain economically viable and biologically interpretable. Molecular changes, in themselves, are not a reason for intervention unless they are

predictors of relevant improvements in health, welfare, reproduction, efficiency or product quality under commercial conditions (Campos *et al.*, 2026; Afruza, 2026).

Development of biomarkers is key for translation. Effective markers need to be assessable in accessible matrices, consistent between laboratories, and predictive when tested in different breeds and environments. Possible candidates include vitamin levels, mineral status enzymes, homocysteine, methionine cycle metabolites, S-adenosylmethionine-derived ratios, oxidative biomarkers, target-specific DNA methylation and circulating microRNAs. One marker is certainly insufficient to capture the overall nutritional status. Composite panels may perform better, but they need independent verification and well-defined reference intervals. They must also distinguish the correction of deficiency from overexposure and transitory metabolic response from permanent developmental programming prior to guiding routine feeding decisions (Cuparencu *et al.*, 2024; La Barbera *et al.*, 2024).

Epigenetic programming raises ethical and regulatory issues. Interventions should enhance health and fitness of the animals, not just maximally produce at the cost of animal welfare and physiological buffer. Statements of transgenerational enhancement demand especially robust evidence as effects in descendants may be due to direct exposure of the fetus and its developing germ cells. Real inheritance must be shown in generations not directly exposed to the original insult. Mineral toxicity, excretion to the environment and interactions with food safety for humans also need to be addressed. The responsible use is informed by conservative interpretation, transparent communication, and coherence among molecular endpoints, animal welfare and sustainable use of resources (Van Otterdijk and Michels, 2016).

11. Research Priorities and Future Perspectives

In the future, research should determine dose, form and timing combinations that result in the outcome of predictable risk or benefit without compromising mineral homeostasis. Studies should compare deficiency, adequacy and supplementation, rather than just two arbitrary levels of inclusion. Interventions in pregnancy need to be subdivided into preconception, placentation, organogenesis, and late fetal growth. Male and female progeny must be observed as sex-specific placental and endocrine alterations may lead to divergent epigenetic results. Cross-breed and cross-environment testing will reveal whether the results hold true in a more general sense. Commercial-scale trials should in the end assess life-time production, disease incidence, reproduction, survival and economic return rather than depend wholly on early molecular end-points (Meesters *et al.*, 2024).

New technology will provide even better mechanistic resolution. Whole-genome bisulfite sequencing, long-read methylation detection, chromatin accessibility assays, histone profiling, single cell sequencing and spatial transcriptomics can be applied to identify nutrient-responsive regulatory elements and cell types. Stable isotope tracers can be used to measure flow of carbon units through folate, methionine and transsulfuration pathways. Metagenomics and metabolomics identify microbial influences on host methyl metabolism. Together with causal modelling and machine learning these data may yield predictive signatures, however there is a need to compare these complex algorithms to simpler models and independent validation is recommended. Translational potential to feeding practice should remain a core consideration in the biology (Wen *et al.*, 2026).

Distinguishing between adaptive plasticity and maladaptive programming is a key importance. Epigenetic variation may assist animals to survive predicted environments, be a form of compensation for nutritional stress, or simply be a record of cellular damage. Challenge experiments with heat, infection or mild nutrient restriction may demonstrate whether programmed individuals are more robust. Reversibility should be studied as postnatal nutrition may rescue adverse effects of prenatal nutritional status and/or exacerbate these. It would be important for research to determine whether selective repair in later windows can restore metabolic function without adding to the dysmetabolism. This innovation could propel nutritional epigenomics from a descriptive association to a usable science of prevention, reclamation and robust livestock production (Swanson *et al.*, 2026).

12. CONCLUSION

Micronutrients bridge nutrition with gene regulation in ruminants via one-carbon metabolism, redox status, mitochondrial function, endocrine signalling and chromatin-modifying enzymes. Epigenetic pathways are affected by folate, vitamins B₁₂, B₂ and B₆, zinc, selenium, iron, copper, manganese, and iodine, and the supply of methyl groups is modulated by choline, betaine, and methionine. These are influenced by such as rumen fermentation, nutrient interactions, tissue partitioning, physiological need and exposure during critical developmental windows. Thus, nutritional epigenomics offers a powerful lens through which to view long-lasting alterations in growth, immunity, reproduction, lactation, metabolic efficiency and environmental sensitivity. But epigenetic variations should not be deemed good, causal or heritable automatically. Translating these to practice will need integrated nutrient, metabolic, molecular and life-time phenotypic measurements enabled by strong longitudinal studies, tissue-specific analyses and independent validation to design feeding strategies that optimise animal resilience, productivity, welfare and sustainability.

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