

One-Carbon Metabolism in Indian Dairy Cattle: Nutritional Regulation of Methyl Donor Availability, Immunometabolism, Liver Health and Epigenetic Programming

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ABSTRACT

One-carbon metabolism (OCM) is an interconnected biochemical network that integrates methionine, choline, folate, vitamin B12 and related nutrients with transmethylation, remethylation, transsulfuration, phospholipid synthesis, nucleotide metabolism and cellular redox control. The system is especially relevant to dairy cattle during the transition from late gestation to early lactation, when dry matter intake often falls while requirements for glucose, amino acids, lipid transport, immune function and milk synthesis increase. Inadequate methyl-donor supply may constrain hepatic phosphatidylcholine synthesis and triglyceride export, while methionine availability also influences protein synthesis, antioxidant capacity and immune-cell function. The present review develops an Indian perspective by linking one-carbon nutrition with the diversity of cattle and buffalo production systems, fluctuating feed-resource availability, crop residues, oilseed cakes, agro-industrial by-products, seasonal heat stress and the metabolic risks observed around calving. Particular attention is given to the methionine and folate cycles, the choline-betaine pathway, transsulfuration, immunometabolism, liver lipid handling, reproduction and

maternal-fetal programming. Recent mechanistic, metabolomic, molecular and meta-analytic evidence is integrated, including work published through 2026. Indian evidence from Kankrej cows, Surti buffaloes, Murrah buffaloes and Sahiwal transition cows is discussed alongside international dairy-cattle studies. The review argues that the next phase of research should move from single-nutrient supplementation toward systems-level assessment of bioavailable methyl-donor supply, rumen protection, metabolic phenotype, heat stress, breed or species effects and epigenetic outcomes. This framework could support more precise and context-specific methyl-donor nutrition for Indian dairy cattle and buffaloes.

INTRODUCTION:

One-carbon metabolism refers to a network of reactions that transfers single-carbon units among folate derivatives, methionine-cycle intermediates and related metabolites. In animal tissues, these reactions support methylation, amino-acid interconversion, nucleotide synthesis, polyamine metabolism, phospholipid turnover and antioxidant defense. Methionine occupies a central position because its conversion to S-adenosylmethionine (SAM) provides a universal methyl donor, whereas homocysteine can be remethylated to methionine through folate- and vitamin-B12-dependent methionine synthase or through the betaine-dependent pathway in tissues expressing betaine-homocysteine methyltransferase. Homocysteine can alternatively enter transsulfuration and contribute to cysteine and glutathione formation (Clare *et al.*, 2019; McFadden *et al.*, 2020).

The importance of OCM becomes especially visible during the periparturient period. Dairy cows experience profound changes in endocrine signals, feed intake, tissue nutrient partitioning and immune activity around calving. Reduced intake combined with rapidly increasing milk synthesis drives negative energy balance, adipose lipolysis and

increased hepatic uptake of non-esterified fatty acids. When oxidation and export capacity are insufficient, hepatic triacylglycerol accumulates and metabolic stress increases. The liver must therefore coordinate glucose production, fatty-acid oxidation, lipoprotein assembly, protein turnover, inflammatory signaling and antioxidant defense at the same time (Drackley, 1999; Coleman *et al.*, 2021).

Methyl donors are relevant to this adaptation for several reasons. Choline contributes directly to phosphatidylcholine production and can provide methyl groups after conversion to betaine. Methionine is both a proteinogenic amino acid and a precursor of SAM. Folate carries one-carbon units, while vitamin B12 is required for the transfer of a methyl group from 5-methyl-tetrahydrofolate to homocysteine. Vitamins B6 and B2 and minerals such as cobalt and sulfur also support connected reactions. Consequently, the biological response to a single supplemented nutrient can depend on the status of the entire network rather than on that nutrient in isolation (McFadden *et al.*, 2020; Crouse *et al.*, 2025). The integrated relationship among these nutrients and physiological outcomes is summarized in Figure 1.

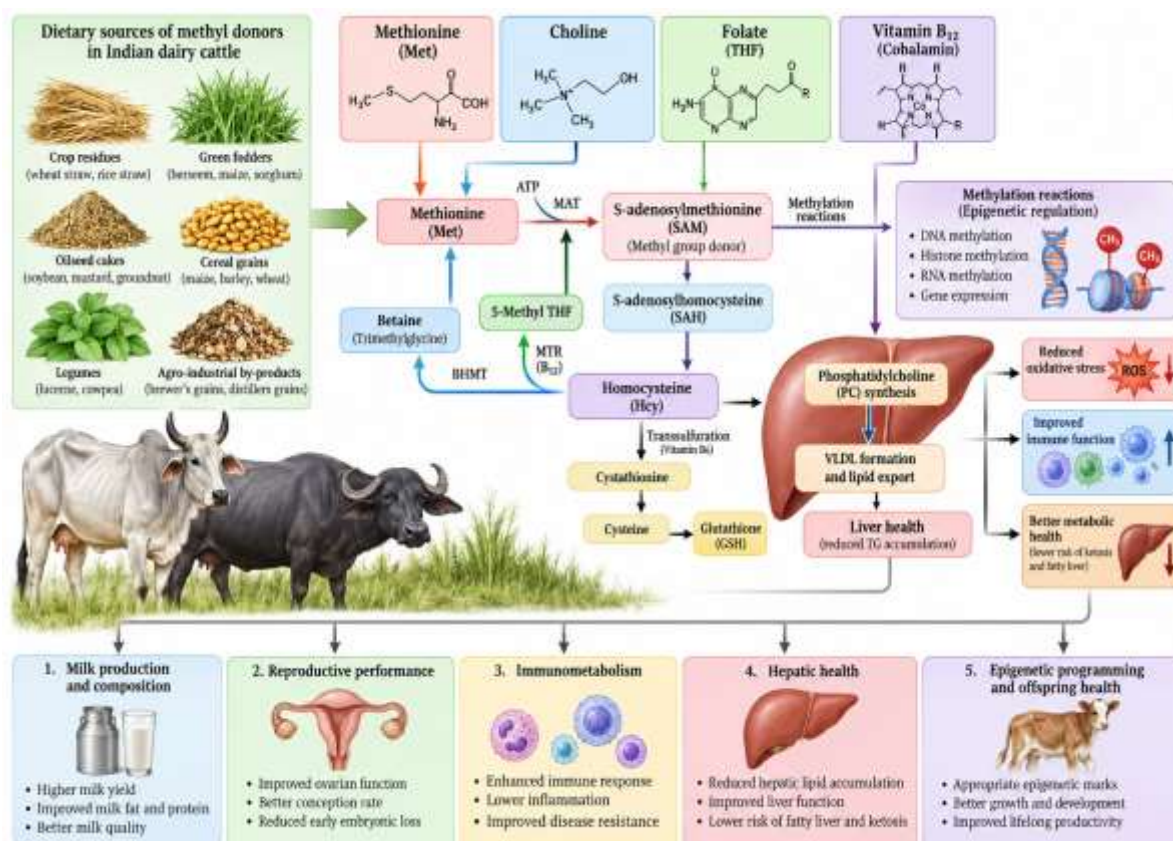


Figure 1. Integrated view of dietary methyl donors and one-carbon metabolism in dairy cattle. The diagram is conceptual and summarizes major pathways and physiological endpoints; arrows indicate biochemical relationships rather than quantitative effect sizes. Source: Authors' synthesis based on McFadden et al. (2020) and Crouse et al. (2025).

India provides a particularly relevant context for examining OCM. The national dairy sector includes indigenous cattle, crossbred cattle and a very large buffalo population, while feeding systems range from intensive mixed rations to smallholder diets based heavily on crop residues, green fodder and locally available concentrate ingredients. The Department of Animal Husbandry and Dairying reported total milk production of 247.87 million tonnes in 2024-25, with cattle and buffaloes together contributing the majority of national milk output (DAHD, 2025). At the same time, ICAR has continued to highlight seasonal and regional fluctuations in feed and fodder supply, including shortages of green and dry fodder (ICAR, 2026). These features mean that the metabolic value of a methyl donor cannot be separated from the basal diet, rumen

degradability, nutrient density, production stage and environmental stress of the animal. The major Indian production-system determinants discussed here are synthesized in Figure 2.

1. Literature approach and scope

This article is a narrative, evidence-integrating review rather than a formal systematic review. Literature was identified from PubMed, ScienceDirect/Elsevier journals, Springer, open-access animal-science journals, ICAR e-pubs and Indian government sources, with emphasis on mechanistic studies, dairy-cattle trials, Indian cattle and buffalo experiments, metabolomics or molecular studies, and systematic reviews/meta-analyses published through September 2026. Seminal work published before 2019 was retained when it

established the biochemical basis of OCM or the biology of the transition period. The synthesis focuses on the nutritional biology of methionine, choline, folate and vitamin B12, with supporting discussion of betaine, vitamins B6/B2, transsulfuration, hepatic lipid

metabolism, immune function, reproductive biology and developmental programming. Table 1 provides a concise map of the principal one-carbon nutrients, pathways and physiological functions used throughout this review.

Table 1. Major one-carbon nutrients and their principal functions in dairy cattle. Source: Compiled and adapted from McFadden et al. (2020) and Crouse et al. (2025).

S. No.	Nutrient	Primary role	Main pathway	Major relevance
1	Methionine	Proteinogenic amino acid and precursor of SAM	Methionine cycle; transsulfuration	Milk protein synthesis, methylation, antioxidant defense
2	Choline	Structural phospholipid nutrient and methyl-donor precursor via betaine	Choline → betaine; phosphatidylcholine synthesis	Hepatic lipid export, membrane integrity, methyl balance
3	Folate (B9)	Carrier of activated one-carbon units	Folate cycle; 5-methyl-THF formation	DNA synthesis, methylation, cell proliferation, fetal development
4	Vitamin B12	Cofactor for methionine synthase	5-methyl-THF + homocysteine → methionine	Remethylation, methyl balance, cellular metabolism
5	Vitamin B6	Cofactor for transsulfuration reactions	Homocysteine → cystathionine → cysteine	Glutathione production and redox control
6	Riboflavin (B2)	Precursor of FAD needed by MTHFR and related enzymes	Folate-cycle reactions	Supports methyl-group regeneration
7	Betaine	Methyl donor and osmolyte	BHMT-mediated remethylation	Methionine regeneration and cellular osmoprotection

2. Biochemical architecture of one-carbon metabolism

2.1 Methionine cycle and SAM-dependent methylation

Dietary or metabolically regenerated methionine is activated by methionine adenosyltransferase to form SAM. SAM transfers its methyl group to numerous acceptors, generating S-adenosylhomocysteine (SAH), which is subsequently hydrolyzed to homocysteine. The cellular SAM:SAH relationship is therefore a functional indicator of methylation potential: increased SAH can

inhibit many methyltransferases, while adequate regeneration of methionine helps sustain methyl transfer. In a lactating animal, methyl-group utilization is extensive because methylated compounds are required for membrane phospholipids, creatine synthesis, gene regulation and other anabolic reactions (Clare et al., 2019; McFadden et al., 2020).

Methionine also has a second major fate. When homocysteine is directed toward transsulfuration, cystathionine and cysteine are produced, providing substrate for glutathione synthesis. This gives methionine a direct

connection to antioxidant defense that is independent of protein synthesis. Studies in transition cows have therefore associated greater methionine supply with altered glutathione status and improved indicators of innate immune-cell function (Zhou *et al.*, 2016a; Batisel *et al.*, 2018).

2.2 Folate cycle and vitamin B12-dependent remethylation

Folate enters cellular metabolism after conversion to tetrahydrofolate (THF), which accepts and transfers one-carbon units in different oxidation states. Serine hydroxymethyltransferase generates 5,10-methylene-THF, while methylenetetrahydrofolate reductase produces 5-methyl-THF, the folate form used by methionine synthase. Methionine synthase is vitamin-B12-dependent and couples the folate and methionine cycles by transferring a methyl group from 5-methyl-THF to homocysteine. Folate therefore does not simply act as a vitamin in isolation; it functions as part of a network that determines the availability of methyl groups and supports nucleotide synthesis as well as methylation (McFadden *et al.*, 2020).

In ruminants, microbial synthesis and ruminal transformation complicate interpretation of B-vitamin nutrition. Ruminal microbes can synthesize folates and cobalamin, but the amount that reaches the small intestine, the extent to which endogenous and microbial production satisfies post-ruminal needs, and the animal's requirement under different physiological conditions are not fully resolved. Consequently, the response to supplemental

folate or B12 can vary with diet, animal stage, microbial supply and concurrent methionine status. This uncertainty is a central reason to assess the whole one-carbon network instead of using single-biomarker approaches (McFadden *et al.*, 2020; Crouse *et al.*, 2025).

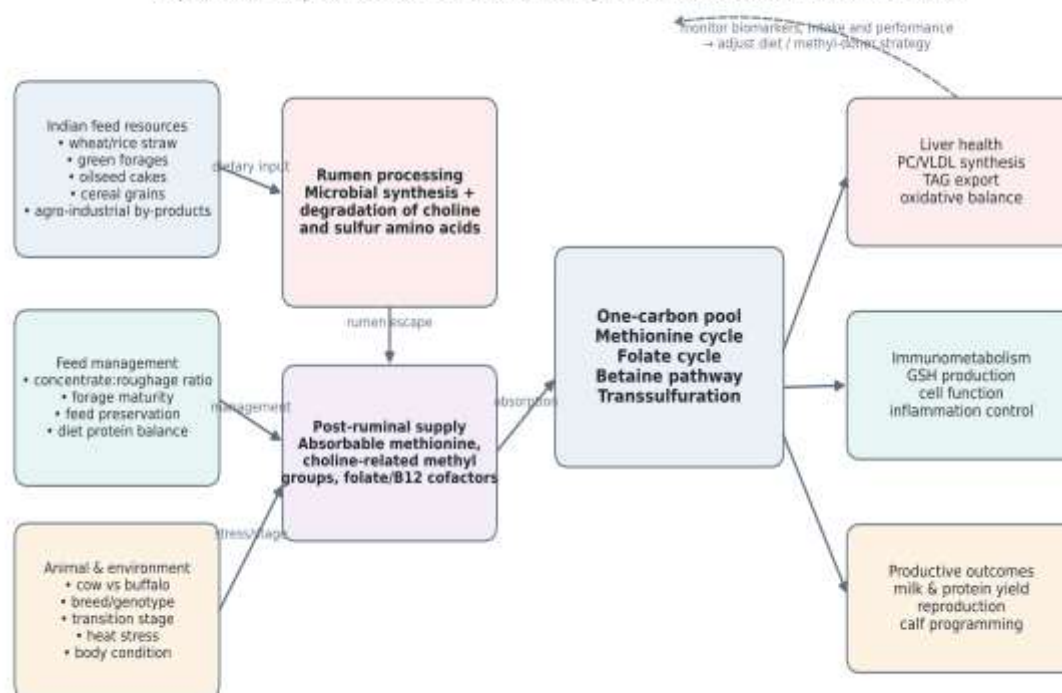
2.3 Choline-betaine pathway

Choline has both structural and methyl-donor functions. It is incorporated into phosphatidylcholine and other membrane phospholipids and can be oxidized to betaine, which donates a methyl group for remethylation of homocysteine through betaine-homocysteine methyltransferase. Phosphatidylcholine is also important for lipoprotein assembly and hepatic export of triglycerides. Thus, choline has a distinctive connection with liver lipid handling that complements its role within OCM (Shahsavari *et al.*, 2016; McFadden *et al.*, 2020).

However, dietary choline is susceptible to ruminal degradation. This is why rumen-protected choline formulations have become a major research tool: they are intended to deliver more choline to the small intestine than unprotected dietary choline. The magnitude of the response depends on protection technology, dose, diet, baseline choline supply and the status of other methyl donors. Recent meta-analysis supports a dose-responsive increase in milk yield around moderate total choline chloride doses, but the authors caution that nominal dietary dose is not equivalent to absorbed choline because protection and diet characteristics differ among studies (Ghaffari *et al.*, 2025).

Indian production determinants of one-carbon nutrient supply and utilization

A systems view linking feed resources, rumen losses, animal stage and environmental stress with metabolic outcomes



Potential research gap: quantify bioavailable methyl-donor supply from Indian diets under seasonal, breed and heat-stress conditions.

Figure 2. Indian production-system determinants of one-carbon nutrient supply and utilization. The figure emphasizes interactions among feed resources, rumen processing, animal stage, environmental stress and downstream metabolic outcomes. Source: Authors' synthesis based on Pawar et al. (2023), Rathwa et al. (2023), Sahu et al. (2026), ICAR (2013, 2026), and Crouse et al. (2025).

3. One-carbon metabolism during the transition period

The transition period is conventionally defined as approximately three weeks before to three weeks after calving and is characterized by major metabolic adaptation (Drackley, 1999). Dry matter intake can decline immediately before calving while lactation demand rises sharply after calving. This mismatch creates negative energy balance, increases adipose lipolysis and elevates circulating NEFA. The liver receives a large flux of fatty acids and must partition them between oxidation, ketogenesis and esterification/export. If triglyceride export is insufficient, hepatic lipodosis develops. The same metabolic stress is associated with oxidative and inflammatory responses, making immune competence and

nutrient partitioning tightly connected (Coleman et al., 2021; Kang et al., 2025).

OCM becomes relevant because the transition cow simultaneously increases demand for phospholipid synthesis, lipoprotein assembly, protein synthesis, antioxidant production and methylation-related processes. The methionine cycle supplies SAM, while choline supports phosphatidylcholine synthesis and betaine-mediated remethylation. Transsulfuration supplies cysteine for glutathione. Folate and B12 support the remethylation machinery. These pathways compete and cooperate for common intermediates, so a bottleneck at one point can alter flux elsewhere. The concept of methyl-donor “balance” is therefore more useful than the simple concept of an isolated deficiency (McFadden et al., 2020; Coleman et al., 2021).

4. Methionine: beyond a limiting amino acid

Methionine is traditionally discussed in dairy nutrition as an essential amino acid whose digestible or metabolizable supply can limit milk-protein synthesis. Its one-carbon significance, however, extends beyond protein incorporation. Methionine is the precursor to SAM, and methylation is linked to creatine synthesis, phosphatidylcholine synthesis and regulation of gene expression. It also provides sulfur for transsulfuration and glutathione synthesis. Therefore, an increase in post-ruminal methionine supply may affect both productive and non-productive pathways (Zhou *et al.*, 2016a; Chiogna Junior *et al.*, 2021).

Studies comparing protected methionine and choline illustrate why pathway context matters. Zhou *et al.* (2016b) reported that rumen-protected methionine improved several immunometabolic indicators relative to choline during the periparturient period, including liver glutathione and leukocyte function. Another trial reported greater milk, energy-corrected milk and milk-protein yield with protected methionine, whereas choline alone did not produce the same pattern of responses (Zhou *et al.*, 2016b). These findings do not imply that methionine is universally superior to choline; rather, they emphasize that the physiological roles of the two nutrients overlap but are not identical.

More recent work in tropical dairy systems indicates that methionine supplementation can support production when the basal diet is otherwise adequate. A 2021 trial in high-producing dairy cows in a tropical environment found that rumen-protected methionine increased milk yield and milk-protein output and increased circulating methionine (Chiogna Junior *et al.*, 2021). In a low-protein strategy, protected methionine also supported milk production and nutrient utilization while allowing dietary crude protein

to be reduced (Zhang *et al.*, 2022). These results are relevant to India because lower-protein diets with adequate essential amino-acid balance may reduce nitrogen excretion, but this approach must be evaluated against the amino-acid profile of local feeds and the economics of protected amino-acid products.

5. Choline: hepatic lipid handling and methyl economy

Choline has attracted considerable interest in transition-cow nutrition because of its role in phosphatidylcholine synthesis and hepatic lipid export. Early-lactation cows receive a large influx of NEFA into the liver; the ability to package triglyceride into VLDL is important for preventing excessive lipid retention. Choline contributes to phosphatidylcholine synthesis through both the Kennedy pathway and the methylation of phosphatidylethanolamine. Because betaine produced from choline can also remethylate homocysteine, choline can reduce the pressure on methionine to serve purely as a methyl donor (McFadden *et al.*, 2020; Zenobi *et al.*, 2018).

The evidence base has become more nuanced with newer dose-response analyses. Ghaffari *et al.* (2025) synthesized 30 studies and found significant dose-response relationships for DMI, milk yield and some milk-component responses, with estimated peaks around 13-14 g/d of choline chloride for DMI and 13 g/d for total milk yield. A separate 2026 metabolic meta-analysis of 29 studies found non-linear responses in prepartum cholesterol, triglycerides and postpartum AST, while emphasizing that dose-response estimates based on nominal choline chloride should not be interpreted as absorbed choline. The authors also highlighted inconsistent reporting of methionine supply, DMI and protection technology (Rezaei-Ahvanooei *et al.*, 2026). Together, these analyses support a precision-nutrition perspective: the useful “dose” is

likely to depend on the nutrient context and bioavailability rather than a universal number.

6. Folate, vitamin B12 and the often-overlooked cofactors

Folate and vitamin B12 are sometimes overshadowed by methionine and choline because the latter two are often supplemented experimentally at relatively conspicuous levels. Yet the methionine synthase reaction directly depends on both folate-derived 5-methyl-THF and cobalamin. Folate is also consumed in nucleotide synthesis and other biosynthetic reactions. When one-carbon demand increases, partitioning between remethylation and other folate-dependent processes becomes important. Therefore, a methionine supplementation strategy may not operate optimally if folate or B12 is inadequate or if methyl-group demand is shifted toward competing pathways (McFadden *et al.*, 2020).

The one-carbon network also contains “supporting” nutrients. Riboflavin provides FAD for enzymes including MTHFR; vitamin B6 supports serine hydroxymethyltransferase and transsulfuration; cobalt is required for ruminal microbial vitamin B12 synthesis; sulfur is needed for sulfur-amino-acid metabolism. In practice, this means that methyl-donor nutrition should be interpreted together with general micronutrient status and rumen function. A research diet that raises methionine or choline but remains poorly balanced for cofactor supply can produce a misleadingly small response or shift flux into alternative pathways (Crouse *et al.*, 2025). Table 2 summarizes the main Indian feed-resource categories and the practical factors that can modify OCM nutrient supply and response.

Table 2. Indian feed-resource categories relevant to one-carbon nutrition and the major factors that can modify response. Source: Authors’ synthesis based on ICAR (2013, 2026), McFadden *et al.* (2020), and Crouse *et al.* (2025).

S. No.	Feed/resource	Potential OCM relevance	Important limitation
1	Cereal grains (maize, wheat, barley)	Protein and methionine-containing substrates; variable choline	Energy density; inclusion level; processing
2	Oilseed cakes (soybean, groundnut, mustard)	Protein and sulfur amino acids; some choline	Amino-acid profile, heat treatment, fat content
3	Green forages and legumes	Folate-related vitamins and degradable protein	Season, maturity and preservation
4	Wheat/rice straw and other residues	Large DM source but low nutrient density	High fibre; low crude protein; variable digestibility
5	Agro-industrial by-products	Potentially useful protein, fibre, lipids and bioactives	Batch variation, anti-nutritional factors, mycotoxin risk
6	Silage/fermented forages	Energy and fermented substrate; indirectly affects microbial metabolism	Fermentation quality, dry matter and storage losses
7	Mineral-vitamin premixes	Support B-vitamin/mineral cofactors	Actual bioavailability and mixing uniformity

7. One-carbon metabolism and liver health

The liver is the primary integrator of the metabolic consequences of transition-cow nutrition. During negative energy balance, NEFA uptake increases, and hepatocytes increase fatty-acid oxidation and ketogenesis. At the same time, triglyceride must be exported in lipoproteins or stored in the cell. Phosphatidylcholine is essential for lipoprotein assembly, creating a mechanistic rationale for choline supplementation when hepatic lipid load is high. Methyl-donor supplementation can also alter the circulating lipidome and hepatic lipid trajectories even when DMI or milk yield changes little (Zang *et al.*, 2019).

In a controlled study of periparturient Holstein cows, co-supplementation with methionine, choline, betaine, riboflavin and vitamin B12 suppressed the progression of hepatic lipid accumulation and changed plasma triacylglycerol and cholesteryl-ester profiles, although it did not increase DMI or milk yield (Zang *et al.*, 2019). The significance of this result is conceptual: a nutritional intervention

can affect metabolic resilience without producing a large production response. Liver tissue, blood metabolites and lipidomic markers may therefore be more sensitive outcomes for evaluating OCM than milk yield alone.

Recent molecular work further supports this systems view. Potts *et al.* (2023) found that protected choline and methionine altered circulating choline metabolites, amino acids and hepatic expression of genes involved in one-carbon and lipid metabolism. Complementary hepatic metabolomics and proteomics studies have also shown that transition to early lactation changes gluconeogenesis, fatty-acid oxidation, amino-acid metabolism, AMP-activated protein kinase signaling and PPAR-related pathways. These datasets illustrate the growing opportunity to combine nutritional trials with multi-omics phenotyping (Potts *et al.*, 2023; Sun *et al.*, 2023). The metabolic pressures described in this section and their nutritional implications are consolidated in Table 3.

Table 3. Transition-period metabolic pressures and potential links with one-carbon nutrition.
Source: Authors' synthesis based on Drackley (1999), Coleman *et al.* (2021), McFadden *et al.* (2020), Ghaffari *et al.* (2025), and Rezaei-Ahvanooei *et al.* (2026).

S. No.	Metabolic pressure	Mechanism	Potential consequence	Nutritional implication
1	Negative energy balance	Adipose lipolysis → NEFA influx	Higher risk of ketosis and hepatic TAG accumulation	Support intake, glucose precursors and lipid export; monitor NEFA/BHB
2	High hepatic lipid load	Need for phosphatidylcholine/VLDL assembly	Fatty liver, impaired hepatic function	Choline; adequate methyl-donor balance; body-condition management
3	High oxidative demand	Greater need for glutathione and antioxidant systems	Oxidative stress and immune dysfunction	Methionine via transsulfuration; balanced B-vitamin/cofactor supply
4	Inflammatory activation	Nutrient partitioning toward immune function	Mastitis/metritis risk and reduced performance	Integrated energy, amino-acid and methyl-donor nutrition

S. No.	Metabolic pressure	Mechanism	Potential consequence	Nutritional implication
5	Low DMI	Reduced nutrient intake despite high requirements	Methyl-donor and amino-acid supply may become limiting	Highly available post-ruminal nutrient supply; precise formulation

8. One-carbon metabolism and immunometabolism

The transition period is associated with transient immune dysfunction and systemic inflammatory activation. Nutrient partitioning changes as glucose, amino acids and fatty acids are directed toward milk synthesis, while leukocytes and other immune cells also require substrates for proliferation, membrane turnover, redox control and cytokine production. OCM intersects these processes through protein and nucleotide synthesis, methylation and glutathione production. Methionine-derived cysteine can contribute to glutathione, whereas methylation reactions can influence transcriptional programs in immune cells (Coleman *et al.*, 2021).

Zhou *et al.* (2016a) reported greater hepatic total and reduced glutathione and improved neutrophil phagocytosis and oxidative burst after protected methionine supplementation compared with choline in a peripartal-cow experiment. Indian evidence is concordant with this mechanistic direction. Rathwa *et al.* (2023) studied 27 pregnant Surti buffaloes and found that protected methionine alone and protected methionine plus protected choline increased glutathione, superoxide dismutase and total antioxidant status and decreased lipid peroxidation; combined supplementation also reduced TNF-alpha and haptoglobin and improved in-vitro neutrophil phagocytic activity and lymphocyte proliferation. These findings are particularly valuable because buffalo physiology and production environments are underrepresented in the international OCM literature.

Choline may also influence immune and reproductive health indirectly through improved metabolic status. In a 2024 study, rumen-protected choline altered the vaginal discharge microbiome and reduced *Fusobacterium* abundance after calving, while also improving lactation performance. Although causality between choline and uterine microbiome is not yet established, the findings open a broader question: can nutritional modulation of OCM influence the host-microbiome interface in ways that change disease susceptibility? This hypothesis deserves controlled trials in Indian cattle and buffaloes (Marques *et al.*, 2024).

9. Reproduction, methyl donors and developmental programming

Reproduction depends on adequate nutrient supply well before peak lactation begins. Folate, B12, methionine and choline participate in cell division, nucleotide synthesis, membrane formation and methylation, making them relevant to oocyte quality, embryo development and placental function. Maternal nutrition during the periconceptual and early-gestation window can influence the developmental trajectory of the embryo and placenta; these effects are increasingly interpreted through the lens of developmental programming and epigenetics (Caton *et al.*, 2020).

Recent cattle studies provide direct biochemical evidence. Syring *et al.* (2024) showed that combined OCM supplementation increased maternal serum B12, folate and several maternal liver metabolites and altered metabolites in fetal fluids and tissues during early gestation. Safain *et al.* (2024) further

showed that nutrient restriction and OCM supplementation changed the fetal-liver metabolome, with effects in amino-acid, vitamin/cofactor, carbohydrate and energy pathways. Importantly, changes in metabolite abundance do not automatically prove a beneficial phenotypic outcome. The fetus appears capable of buffering some maternal changes, which means that future research should measure both maternal biomarkers and offspring phenotypes rather than assuming that a higher circulating methyl donor is equivalent to better developmental programming.

The epigenetic dimension is particularly important. SAM supplies methyl groups for DNA and histone methyltransferases, creating a biochemical route by which maternal nutrient status can influence gene regulation. The 2026 critical review by Guo *et al.* summarized evidence that maternal methionine status can influence offspring development through DNA methylation, histone modification and broader epigenetic networks. For cattle, Crouse *et al.* (2025) described evidence that maternal OCM manipulation can alter metabolic programming across the production cycle, while also emphasizing that physiological stage, diet, genotype, protection status and delivery method change the response. The practical implication is that OCM is potentially a developmental signal, not merely a short-term production supplement.

10. OCM, epigenetics and precision animal nutrition

The concept of precision nutrition becomes more meaningful when methyl-donor supplementation is connected to individual metabolic phenotype. Two cows receiving the same dose can have different responses because of differences in DMI, parity, body condition, milk yield, rumen degradation, baseline methionine status, inflammation, liver lipid burden or heat load. Molecular

heterogeneity adds another layer: expression of enzymes in OCM and lipid metabolism is dynamic across tissues and physiological stages. Precision nutrition therefore requires the measurement of intermediate biomarkers, not simply the final production trait (Coleman *et al.*, 2021; Crouse *et al.*, 2025).

Potential biomarkers include plasma methionine, choline, betaine, homocysteine and BHB; liver triglyceride and phosphatidylcholine-related measures; glutathione redox status; inflammatory proteins such as haptoglobin; and metabolomic patterns. New Indian research in Sahiwal transition cows demonstrates the feasibility of biomarker-based risk prediction under tropical conditions. Sahu *et al.* (2026) used serial serum NEFA, BHBA, glucose and calcium measurements to develop thresholds associated with single or multiple postpartum production diseases. Although this study was not an OCM experiment, it provides an Indian model for the next generation of methyl-donor research: use repeated biochemical phenotyping to identify animals at metabolic risk, then test targeted nutritional interventions.

11. Indian dairy context: why OCM needs an India-specific framework

India's dairy sector is biologically and nutritionally heterogeneous. The national herd includes indigenous breeds, crossbreds and non-descript cattle, while buffaloes contribute a large share of milk. Feeding commonly includes cereal straws, green fodders, crop residues, oilseed cakes, cereal grains, mineral mixtures and a wide range of agro-industrial by-products. Regional feed availability can vary sharply, and ICAR has emphasized seasonal fluctuation in green-fodder availability as a continuing constraint. These conditions can change both the quantity and the chemical form of nutrients entering the rumen and, consequently, the fraction reaching

the small intestine (DAHD, 2025; ICAR, 2026).

An Indian OCM research program should therefore begin with feed characterization. It is not enough to label a ration as “protein adequate.” Methionine supply depends on crude protein source, degradability, microbial protein production and the post-ruminal amino-acid profile. Choline status depends on intrinsic feed choline, ruminal degradation and protection technology. Folate and B12 are linked to microbial metabolism and cobalt availability. In a smallholder setting, seasonal forage maturity and variable inclusion of crop residues may change these inputs from month to month. The first research gap is consequently a quantitative description of bioavailable OCM supply in Indian diets.

The second gap is species and genotype. International dairy evidence is dominated by Holstein cows, yet Indian production includes *Bos indicus* cattle and water buffaloes. Indian experiments already show that protected choline and combined methionine-plus-choline can alter productivity and immunometabolic status in Kankrej cattle and Surti buffaloes

(Pawar *et al.*, 2023; Rathwa *et al.*, 2023). These studies are important proof-of-concept but are still small and geographically concentrated. Larger multi-location trials are needed to determine whether responses depend on breed, buffalo versus cattle physiology, milk yield, parity, basal diet and tropical heat load.

The third gap is environmental interaction. Heat stress can suppress feed intake and alter partitioning of nutrients toward thermoregulation. In Indian summer conditions, the resulting reduction in DMI may intensify negative energy balance during the transition period. It is biologically plausible that this changes the demand for methyl donors and antioxidant substrates, but there is not yet enough evidence to establish a universal heat-stress-specific OCM requirement. Trials should therefore examine OCM under naturally varying temperature-humidity conditions and should measure intake, liver biomarkers, redox status, immune function and production simultaneously. Selected Indian cattle and buffalo studies relevant to OCM and transition-period health are compared in Table 4.

Table 4. Selected Indian evidence relevant to one-carbon metabolism, methyl donors and transition-cow metabolic health. Source: Pawar *et al.* (2023), Rathwa *et al.* (2023), Kumar *et al.* (2019), and Sahu *et al.* (2026).

S. No.	Study	Animal	Intervention/approach	Key finding	Main limitation
1	Pawar <i>et al.</i> , 2023	Kankrej cows	50 g/d rumen-protected choline, -4 to +8 wk	Milk yield and FCM increased; serum glucose increased; TG decreased	Small study (n=20); single location
2	Rathwa <i>et al.</i> , 2023	Surti buffaloes	RPM 10 g/d $\pm$ RPC 50 g/d, -15 to +30 d	Higher GSH/SOD/TAS; lower LPO, TNF-alpha and haptoglobin; better immune-cell responses	Small study (n=27); biomarker-focused
3	Kumar <i>et al.</i> , 2019	Murrah buffaloes	54 g RPC with prill fat	Milk, FCM/ECM and fat yield improved	Combined fat + choline design; not a pure OCM test
4	Sahu <i>et al.</i> , 2026	Sahiwal cows	Serial metabolic profiling, -6 to +7 wk	NEFA, BHBA, glucose and calcium thresholds associated with production-disease risk	Not an OCM supplementation trial

S. No.	Study	Animal	Intervention/approach	Key finding	Main limitation
5	ICAR, 2026	Indian feed-resource system	Strategic assessment of feed resources	Highlights seasonal/declining green-fodder availability as a system constraint	System-level evidence; not methyl-donor specific

12. Practical nutritional considerations for Indian dairy systems

From an applied perspective, methyl-donor supplementation should be viewed as one component of transition-cow management. Body-condition control before calving, gradual diet transition, sufficient physically effective fibre, adequate dietary protein, balanced mineral nutrition, clean water, heat-abatement and disease prevention remain foundational. Choline or methionine cannot compensate for severe underfeeding or poor forage quality. In smallholder systems, the first intervention may be to reduce variability in feed composition and establish consistent ration mixing before introducing specialized supplements.

Rumen protection is a second practical issue. The biological objective of rumen-protected products is to increase post-ruminal delivery; therefore, product dose on the feed label does not equal metabolizable nutrient absorbed by the animal. A protected methionine source can differ in rumen escape and intestinal availability, and choline products differ in protection characteristics. The 2026 choline

dose-response meta-analysis explicitly cautions that dose-response curves based on nominal choline chloride should not be interpreted as absorbed choline. Indian trials should therefore report both nominal dose and, when available, estimates of rumen protection or intestinal delivery.

A third consideration is cost-effectiveness. For Indian farms, the economic value of a methyl-donor strategy should be expressed as cost per unit increase in milk solids, reduced disease incidence, improved feed efficiency or reduced veterinary cost—not milk volume alone. Some interventions may produce their largest value through fewer metabolic disorders or improved resilience rather than through a large immediate milk response. This is especially relevant in indigenous cattle and buffalo production systems where baseline milk yield and diet composition differ from high-input Holstein systems. Table 5 translates these principles into a practical framework for an Indian precision OCM nutrition trial.

Table 5. Suggested framework for an Indian precision OCM nutrition trial. Source: Authors' synthesis informed by Crouse et al. (2025), Potts et al. (2023), and Sahu et al. (2026).

S. No.	Research step	Core measurements	Purpose
1	Define metabolic phenotype	NEFA, BHBA, glucose, BCS, milk yield, liver enzymes	Identify OCM-responsive subgroups
2	Measure baseline OCM	Methionine, choline/betaine, folate, B12, homocysteine	Quantify the degree of methyl-donor limitation
3	Characterize rumen environment	DMI, diet composition, degradable protein, microbial markers	Estimate pre- and post-ruminal supply

S. No.	Research step	Core measurements	Purpose
4	Use targeted supplementation	RPM, RPC, folate/B12 or rational combinations	Test pathway-specific versus network effects
5	Measure liver/redox endpoints	TAG, phospholipid markers, GSH, SOD, LPO, AST/GGT	Link nutritional exposure to mechanism
6	Add immune and reproductive outcomes	Haptoglobin, cytokines, leukocyte function, fertility	Capture whole-animal consequences
7	Add omics when feasible	Metabolomics, transcriptomics, DNA methylation	Reveal mechanistic heterogeneity and programming
8	Evaluate farm economics	Feed cost, disease cost, milk solids, persistence	Translate biology into practical recommendations

13. Current evidence: what is established and what remains uncertain

The evidence for OCM in dairy cattle is strong at the biochemical level but more heterogeneous at the whole-animal level. It is well established that methionine, folate, B12, choline/betaine and related cofactors participate in interconnected pathways. It is also well established that the transition period creates high metabolic demand and that liver lipid accumulation, oxidative stress and inflammation can occur together. The uncertainty lies in how large a functional limitation exists in a given diet, which methyl donor is first limiting, whether supplementation should be individual or combined, and which phenotype best predicts response (McFadden *et al.*, 2020; Coleman *et al.*, 2021).

Choline offers one of the clearest examples. Meta-analytic evidence indicates that rumen-protected choline can improve DMI or milk yield under certain dose ranges, but the newer metabolic meta-analysis shows that the relationship is not simply linear and may differ for cholesterol, triglycerides and AST. Furthermore, studies use different products, levels of choline ion, feeding windows and basal diets. This heterogeneity makes universal dosage statements inappropriate for all Indian cattle and buffaloes. The practical research question is not “what is the best choline dose?”

but “what level of bioavailable choline is optimal for an animal with a defined diet, methionine status, production level and metabolic phenotype?” (Ghaffari *et al.*, 2025; Rezaei-Ahvanooei *et al.*, 2026).

Methionine shows a similar complexity. Its effect on milk-protein synthesis is relatively predictable when it is limiting, but its effects on redox and immune function depend on sulfur-amino-acid partitioning and physiological stage. Co-supplementation can be rational when multiple pathways are under pressure, but combined formulations also make it harder to identify the causal nutrient. This is why future studies should include factorial designs, pathway-specific biomarkers and metabolomic analysis rather than only control versus combined-supplement groups (Potts *et al.*, 2023; Crouse *et al.*, 2025).

14. Emerging frontier: OCM, epigenetics and life-course nutrition

One-carbon metabolism provides a direct biochemical bridge between diet and the epigenome. SAM supplies methyl groups for DNA and histone methyltransferases; folate and B12 influence the regeneration of methionine; choline and betaine can affect remethylation; methionine availability can alter transsulfuration and methylation simultaneously. These pathways therefore have the potential to convert a short-term

nutritional environment into a durable change in gene regulation. The concept is particularly important during embryonic and neonatal development when cell fate, organ growth and metabolic systems are being established (Clare *et al.*, 2019; Caton *et al.*, 2020).

In cattle, recent work shows that maternal OCM supplementation changes concentrations of OCM intermediates in the dam and fetal environment and alters fetal liver metabolite patterns. Crouse *et al.* (2025) reviewed evidence across the production cycle and emphasized developmental programming as a major research direction. The 2026 critical review of maternal methionine further synthesizes evidence that maternal methionine status can influence offspring growth and metabolism through DNA methylation, histone modification and related epigenetic networks (Guo *et al.*, 2026). Yet evidence from animal models should not be automatically translated into cattle management recommendations. The next step is longitudinal livestock research linking maternal diet to measurable methylation changes, calf phenotype, immune competence and lifetime efficiency.

An India-specific epigenetic agenda could focus on indigenous cows and buffaloes exposed to variable prenatal nutrition and heat stress. The question would be whether maternal methyl-donor supply modifies epigenetic signatures that influence calf growth, immune resilience, reproductive development or later metabolic efficiency. Such work could bridge Animal Nutrition with nutrigenomics and livestock genetics while also contributing to precision breeding and management. Importantly, epigenetic findings should be validated functionally; differences in DNA methylation are not necessarily beneficial or causal unless linked to gene expression, phenotype and reproducible developmental outcomes.

15. Rumen microbiome and one-carbon metabolism

The rumen adds another layer of complexity because microorganisms transform dietary protein and synthesize or degrade several B vitamins and methyl-containing compounds. Microbial protein contributes substantially to the amino-acid supply of ruminants, while ruminal degradation can reduce the amount of intact methionine or choline available post-ruminally. Therefore, OCM in a ruminant cannot be understood only from the composition of the formulated diet. The relevant variable is the interaction between diet, rumen microbial ecology, escape from ruminal degradation and intestinal absorption (McFadden *et al.*, 2020; Crouse *et al.*, 2025).

Emerging studies increasingly connect methyl-donor supplementation with microbial endpoints. Choline supplementation has been shown to alter reproductive-tract microbial profiles in postpartum cows, while OCM status itself can influence host metabolism and immune tone. A future research model could integrate rumen metagenomics, metabolomics and host OCM markers to determine whether specific microbial communities predict methyl-donor response. In India, this approach could be especially informative because diets rich in straw, tree leaves, crop residues or agro-industrial by-products may produce very different rumen environments from standardized total mixed rations.

16. Precision one-carbon nutrition and future diagnostic tools

The next generation of OCM nutrition is likely to be phenotype-driven. Instead of supplementing every animal in the same way, animals could be stratified by parity, body condition, DMI, milk yield, NEFA/BHB pattern, liver-risk score and perhaps circulating OCM metabolites. Such stratification would allow targeted use of protected methionine,

choline or combinations. Precision feeding technology could support this approach by collecting daily milk yield, activity, rumination and feed-intake proxies, while handheld or laboratory assays provide metabolic biomarkers (Sahu *et al.*, 2026).

Artificial intelligence and machine learning could eventually integrate these heterogeneous measurements. A predictive system might estimate the probability of metabolic disease, classify the animal's likely limiting pathway and propose a feed adjustment. However, the model would only be as reliable as the underlying data. Indian datasets need breed diversity, buffalo representation, tropical climate records, local feed-resource variation and explicit measurement of supplement bioavailability. A model trained primarily on

Holstein cows in temperate TMR systems should not be assumed to transfer unchanged to smallholder Indian cattle or buffaloes.

Metabolomics is particularly promising because OCM generates measurable intermediates such as SAM, SAH, betaine, dimethylglycine, cystathionine, taurine and glutathione-related metabolites. The 2024 fetal-liver studies and transition-cow multi-omics work demonstrate that these tools can identify pathway shifts not visible in routine blood chemistry. The research opportunity is to discover composite biomarkers that predict disease risk or response before clinical signs emerge. This could enable “metabolic phenotyping” of dairy cows and a shift from reactive therapy to preventive nutrition.

Table 6. Priority research questions for one-carbon metabolism in Indian dairy cattle and buffaloes. Source: Authors' synthesis based on McFadden *et al.* (2020), Crouse *et al.* (2025), Ghaffari *et al.* (2025), Rezaei-Ahvanooei *et al.* (2026), and Indian studies cited in Table 4.

S. No.	Research gap	Key question	Recommended approach
1	Bioavailable OCM supply from Indian diets	Quantify post-ruminal methionine, choline, folate/B12 supply across regional diets	Controlled feeding + rumen escape studies
2	Breed/species effects	Compare indigenous cattle, crossbreds and buffaloes	Large multi-location factorial trials
3	Heat-stress interaction	Test whether heat load changes OCM requirement or response	Repeated THI + OCM biomarkers + production
4	Methyl-donor combinations	Define when combined supplementation is additive, redundant or antagonistic	Factorial designs with metabolomics
5	Early disease prediction	Identify OCM-linked biomarkers before ketosis/ fatty liver	Longitudinal cohort studies
6	Epigenetic programming	Link maternal OCM supply to calf epigenome and phenotype	Maternal intervention + offspring follow-up
7	Microbiome interaction	Identify microbial predictors of OCM utilization	Metagenomics + metabolomics + host biomarkers
8	Farm economics	Quantify return on investment in Indian settings	On-farm trials and partial-budget analysis

CONCLUSIONS

One-carbon metabolism provides a unifying framework for understanding how methionine, choline, folate and vitamin B12 interact with liver function, oxidative balance, immune competence, reproduction and gene regulation in dairy cattle. The transition period exposes the importance of this network because nutrient intake falls at the same time that mammary output, hepatic processing and immune demands rise. Methionine supplies SAM and sulfur for transsulfuration; choline contributes phosphatidylcholine and methyl groups through betaine; folate carries one-carbon units; and B12 couples the folate and methionine cycles. These pathways are functionally connected rather than nutritionally independent.

For India, the main challenge is not a lack of plausible mechanisms but limited context-specific evidence. Indigenous cattle and buffaloes, regionally variable crop-residue diets, fluctuating fodder availability, heat stress and diverse management systems can all alter the response to methyl donors. Indian studies in Kankrej cows, Surti buffaloes and Murrah buffaloes provide encouraging local evidence for changes in production, lipid metabolism, antioxidant status and immune responses, while new Sahiwal work demonstrates the value of repeated metabolic phenotyping. Nevertheless, small sample sizes and single-location studies remain common, and there is still no validated India-wide requirement framework for bioavailable methyl-donor supply during the transition period.

The future of this field is therefore likely to move from single-nutrient supplementation toward precision one-carbon nutrition. Combining feed analysis, rumen and post-ruminal nutrient supply, metabolic biomarkers, targeted metabolomics, liver health indicators, immune phenotyping and epigenetic

measurements could identify which animals are most likely to respond and why. The ultimate objective should be context-specific recommendations that improve metabolic resilience, animal welfare, production efficiency and economic sustainability in Indian dairy systems without assuming that results generated in a single breed, diet or climate apply universally.

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