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REVIEW ARTICLE

Effects of Rumen-Undegradable Protein and Rumen-Protected Amino Acids on Small-Intestinal Microbiota in Sheep with Special Reference to Chinese Hu Breed

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ABSTRACT

In ruminants, dietary crude protein does not directly determine the amino acids available to the small intestine because rumen-degradable protein (RDP) supports microbial synthesis, whereas rumen-undegradable protein (RUP) bypasses fermentation. Together, they define the metabolizable protein (MP) supply. The amino acid profile is delivered to the jejunum and ileum. Adjusting the RUP:RDP balance by using rumen-protected lysine (RP-Lys) and rumen-protected methionine (RP-Met) can improve growth and nitrogen-use efficiency. However, the response depends more on post-ruminal release and bioavailability on protection claims alone. Increasing evidence indicates that segment-specific luminal substrates and epithelial signaling are linked to microbial ecology. These effects include changes in mucosa-associated communities, metabolite networks, and barrier/immune markers. However, many studies rely on rumen or fecal measurements and cannot reliably infer mechanisms in the small intestine. This review synthesizes segment-level evidence relevant to Hu sheep. It also highlights how bioavailability and sampling compartment (digesta vs. mucosa) affect interpretation. In addition, it proposes a diet–substrate–epithelium–microbiota framework to guide future trials. Key gaps include limited causal designs, inconsistent reporting of protected amino acid release/availability, and insufficient integration of nitrogen partitioning with intestinal endpoints. Addressing these gaps will strengthen gut-resilient nutritional strategies in Hu sheep production systems.

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INTRODUCTION

Hu sheep farming is also based on the idea of stimulating rapid growth, feed efficiency, and a stable gut, especially in early life. In that regard, diarrhoea is a constraint that can lower growth and resilience, and nutritional strategies that facilitate intestinal stability have practical significance (Zhong *et al*., 2022).

Hu sheep are an indigenous Chinese sheep breed that is well adapted, early maturing, fertile, and known for high milk yield and rapid growth, making them suitable for intensive lamb production systems (Wang *et al*., 2024). In intensive production systems, diarrhoea is a major health and productivity concern in Hu lambs. Published findings show that diarrhoeic Hu lambs have reduced gut bacterial diversity, altered bacterial composition, and lower serum IgG and IgM concentrations compared with healthy lambs (Zhong *et al*., 2022). Recent studies in Hu lambs also show that optimizing dietary metabolizable protein can improve growth performance, plasma amino acid profiles, and nitrogen-use efficiency, while also altering rumen bacterial populations (Cai *et al*., 2025). Therefore, Hu sheep provide an important model for reviewing the

effects of post-ruminal amino acid supply on jejunal and ileal microbiota, barrier function, and lamb resilience.

Recent studies have demonstrated that various plant-derived bioactive compounds, including essential oils, tannins, and polysaccharides, can significantly enhance growth performance, antioxidant status, immune function, and intestinal microbiota balance in both animals and disease models, thereby improving overall health and nutrient utilization (Chen *et al*., 2023; Liu *et al*., 2023; Wang *et al*., 2024). Because of their nutritional and medicinal benefits, medicinal plants have been well researched (Gilani et al., 2025). Similarly, medicinal plant extracts such as *Astragalus* and *Dendrobium officinale* polysaccharides have been shown to strengthen intestinal barrier integrity and modulate gut microbial composition, contributing to improved metabolic health and disease resistance (Su *et al*., 2023; Chen *et al*., 2025). In ruminants, the dietary crude protein is processed in the rumen. Consequently, what reaches the small intestine is a mixture of microbial true protein, undegraded dietary protein (RUP), and secretions of the body, and not the dietary crude protein itself (Han *et al*., 2022; Zhang *et al*., 2024).

The concept of operational definitions used in the context of protein evaluation models identifies RDP as the fraction of rumen-degradable protein to maintain the growth of microbes and RUP as the fraction to avoid rumen degradation and move to the intestinal location. Microbial protein can represent a major contributor to duodenal protein flow. However, its quantity varies with rumen substrate supply and synchrony. This links rumen management to the nitrogenous substrate landscape presented to the jejunal and ileal segments (Zhang *et al*., 2020).

The jejunum and ileum represent distinct ecological compartments of the ruminant gastrointestinal tract. Intestinal location is a major determinant of microbial community structure, and jejunum–ileum differences are usually smaller than those in the foregut and hindgut regions (Li *et al*., 2025; Zhang *et al*., 2025). Alpha diversity is generally lower in the jejunum and ileum than in other gastrointestinal compartments, reflecting physiological filtering, rapid flow, and close host–microbe proximity (Su *et al*., 2025). Notably, mucosa-associated communities may differ from fecal communities. Studies of the mucosal ileum have highlighted host–microbe metabolic coupling at the mucosal interface. However, evidence on the ileal mucosa in ruminants remains limited. These characteristics make the jejunal and ileal microbiota biologically significant endpoint targets, rather than using feces as a surrogate (Zhang *et al*., 2021a; Li *et al*., 2025). The digestion of feed and disintegrate of nutrients in the rumen is assisted via rumen microbes which produce a variety of hydrolyzing enzymes (Yue et al., 2023). These microbial communities influence fermentation products, nitrogenous substrates, mucosal barriers, and immune signaling at the nutrient absorption interface. Therefore, the jejunal and ileal microbiota are directly relevant to nutrient utilization efficiency and susceptibility to diarrhoea in Hu lamb production.

Nutritional interventions can modify post-ruminal amino acid supply by altering the RUP:RDP ratio or by supplementing rumen-protected lysine and methionine. These changes may alter small intestinal ecological niches by modifying the shape and timing of luminal nitrogenous substrates, intestinal transport, and intestinal barrier biology (Fig. 1) (Zhang *et al*., 2019). Research on Hu lambs has associated diarrhoea with reduced intestinal bacterial diversity. It has also identified biochemical and immunological biomarkers indicating disrupted ecological stability of the small intestine as a direct determinant influencing health in this production environment (Zhong *et al*., 2022). However, findings across studies can vary by intestinal segment (jejunum vs. ileum), sampling niche (digesta vs. mucosa), and baseline diet composition. They can also vary by the release/bioavailability of protected amino acid products, which is not always directly verified. Critically, segment-level (jejunum/ileum) evidence in Hu sheep remains limited compared with rumen or fecal studies. In addition, many 'protected AA' trials do not verify post-ruminal release/bioavailability, which complicates mechanistic comparison. The key question is therefore not only whether supplementation is provided. It is also whether dietary protein degradability and maintained amino-acid supply cause measurable changes in jejunal and ileal microbiota composition, metabolites, and host-microbe interaction, which cause a growth and resilience phenotype (Ji *et al*., 2024).

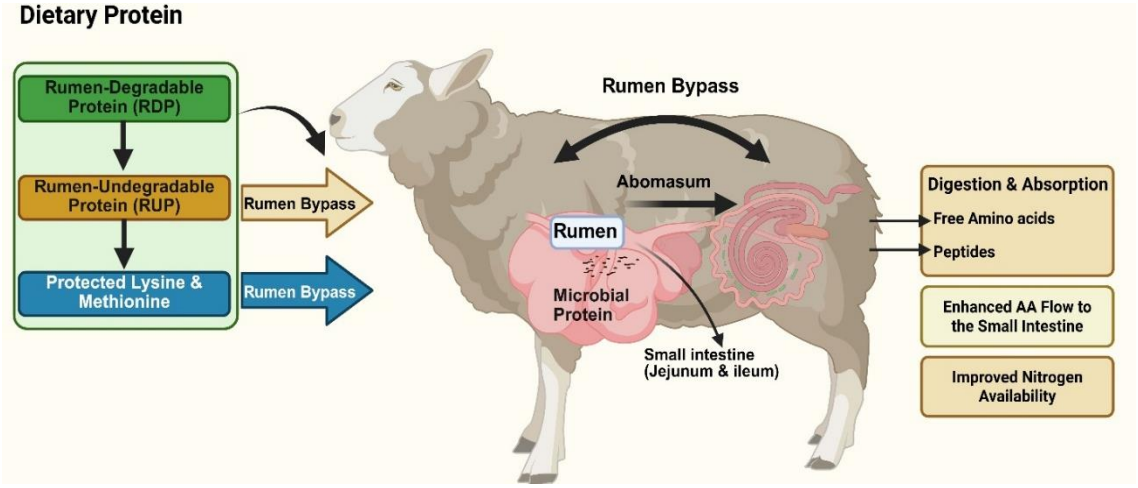


Fig. 1: Rumen-undegradable protein and post-rumen lysine/methionine availability in ruminants. Note: RDP, rumen-degradable protein; RUP, rumen-undegradable protein; AA, amino acid.

Scope and approach: This narrative review is a summary of evidence on the effect of the provision of post-ruminal amino acids on the jejunal and ileal microbiota of sheep. It focuses on Hu sheep and two modifiable levers: the dietary ratio of RUP:RDP, and rumen-protected lysine/methionine. The literature was selected by searching large databases (e.g., PubMed, Web of Science, and Google Scholar) of peer-reviewed articles on the topic of ruminant nutrition, microbiota, and intestinal physiology. Search terms combined concepts for (i) protein degradability and metabolizable protein ("RUP", "RDP", "metabolizable protein", "bypass protein") and (ii) rumen-protected amino acids ("rumen-protected lysine", "rumen-protected methionine", "protected amino acids", "bioavailability"). They also included (iii) intestinal segments and microbiota ("jejunum", "ileum", "mucosa", "digesta", "microbiome", "metabolome", "barrier", "tight junction"). Priority was given to sheep/lamb studies reporting jejunal or ileal outcomes (microbiota composition/diversity, metabolites, morphology, and barrier or immune markers). Studies focused only on feces were used mainly as a supportive context, unless segment sampling was unavailable. Where evidence on Hu sheep was limited, mechanistically relevant data from other small ruminants or cattle were included and clearly labelled as supporting evidence. The reference lists of relevant articles were also screened to capture additional segment-level and bioavailability studies.

Protein Nutrition in Ruminants: RUP and RDP Fundamentals: Protein assessment models state that metabolizable protein, the sum of amino acids absorbed by the small intestine, is based on digestible microbial true protein and digestible undegraded feed protein. It is not based on dietary crude protein itself (Adhikari *et al*., 2022). In a practical system, RDP may be represented as the product of effective degradability and crude protein. RUP is the fraction that is not degraded in the rumen. Together, these measures provide a basis for formulation decisions aimed at manipulating the supply of intestinal amino acids (Klatt, 2019; Guo *et al*., 2024).

However, microbial protein yield is contingent on rumen conditions and can be difficult to predict from forage chemical composition alone. This reinforces that the post-ruminal supply depends on interacting constraints, such as fermentable substrate availability and nitrogen synchrony (Mahboobi *et al*., 2023; Zhang *et al*., 2024). This is significant for the ecology of the small intestine. The jejunum and ileum are exposed to a substrate profile shaped by rumen microbial synthesis, escape protein flow, and endogenous secretions, rather than a simple reflection of dietary crude protein level (Han *et al*., 2022; Zhang *et al*., 2025).

The concept of physiological economics also adds complexity to the idea that a higher intestinal supply would always be associated with peripheral protein accretion. Gut tissues absorb essential amino acids significantly on the first pass and contribute a significant percentage of total body amino-acid flux. This implies strong splanchnic competition for absorbed amino acids (Trommelen *et al*., 2021). This provides a mechanistic basis for distinguishing the two pathways by which RUP manipulations could influence the jejunal and ileal microbiota. First, RUP manipulations may alter luminal nitrogenous substrates entering these segments. Second, they may modify epithelial uptake and turnover demands, thereby shaping the microenvironment at the host–microbe interface (Zhang *et al*., 2024; Urga *et al*., 2025).

Protected Amino Acids: Technology and Bioavailability Challenges

Rumen-protected lysine and methionine: concepts and delivery platforms: Intervention trials in sheep and lambs provide proof of concept that the RUP:RDP ratio is not only theoretical. It can affect performance and nitrogen-use outcomes through changes in metabolizable protein supply (Shen *et al*., 2018; Valizadeh *et al*., 2021; Zheng *et al*., 2024). In growing lambs fed a high wheat-straw diet, increasing the RUP:RDP from 25:75 to 35:65 improved average daily gain, feed efficiency, and digestibility indices. It was also associated with changes consistent with improved nitrogen utilization efficiency and microbial protein proxy signals. In young lambs, factorial comparisons of RUP:RDP ratios at various levels of straw inclusion have reported better indicators of improved digestibility and nitrogen efficiency. These results confirm that the manipulation of degradability can affect the downstream nitrogen economy at intestinal locations (Wang *et al*., 2016; Dorri *et al*., 2021). Overall, the evidence base suggests that the optimum RUP:RDP ratio should be treated as relative rather than absolute. The realized biological response depends on effective ruminal degradability, rumen outflow, microbial protein synthesis and escape-protein flow, rather than the formulated ratio alone (Davila *et al*., 2013; Valizadeh *et al*., 2021; Zhang *et al*., 2024). In low-protein diets, Protein solubility was graded in sheep to enhance energy intake and intestinal nitrogen absorption. It was also connected with modifications in fecal microbiome and metabolome phenotypes linked to decreased fecal reactive nitrogen emissions. These results indicate that protein fractionation is another lever, that is, the one that determines the downstream microbial metabolism in CP reduction strategies (Zhang *et al*., 2025).

Effects of Protected Lysine and Methionine on Small-Intestinal Microbiota: Rumen-protected lysine and methionine focus on maintaining supplemented amino acids by ruminal fermentation and releasing them post-ruminally, providing absorbable substrates at levels sufficient to alter host physiology. Nevertheless, rumen protection is mechanistically multi-component, but not a binary term (Fig. 2) (Fleming *et al*., 2019; Fagundes, 2021). In all the assessments, the three domains are repeated: ruminal stability, intestinal release and digestibility, and whole animal bioavailability, which combine losses and limitations between ingestion and absorption (Menchu, 2019; Räisänen *et al*., 2025). There is evidence that focuses on the plasma appearance measurements, in which apparently significant ruminal stability can result in relatively small relative bioavailability. This highlights why the term bypass cannot be mistaken for the term usable lysine without bioavailability statistics (Fagundes, 2021). Wider generalizations also indicate the inconsistency of bioavailability estimations. Also, they indicated that such estimates cannot be stable without standard procedures and strict designs. It warns against treating the claims of the

substrate supply literally without any explicit and dependable evidence of bioavailability (Huhtanen and Ahvenjärvi, 2022; Guo *et al*., 2024; Wang *et al*., 2025).

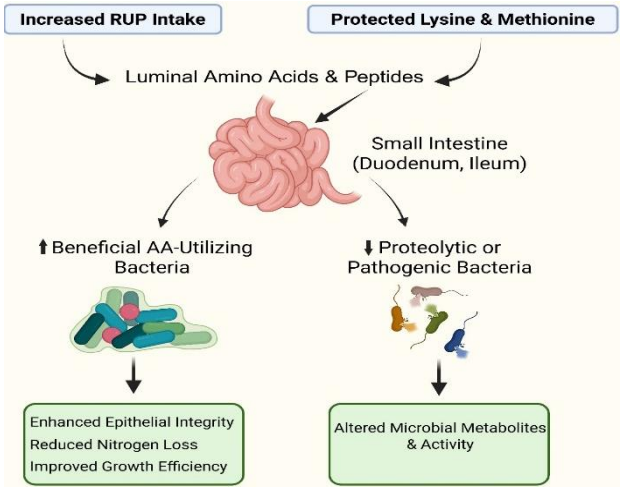


Fig. 2: Effects of rumen-undegradable protein and protected lysine/methionine on small intestinal microbiota. Note: RUP, rumen-undegradable protein; AA, amino acid.

Families of technology that are present in the evidence base are lipid or wax-matrix encapsulation and derivative methods. They are supposed to modify the structure of molecules and decrease the ruminal catabolism (Fleming *et al*., 2019; Inô *et al*., 2024; da Silva Batista *et al*., 2025; Feitosa *et al*., 2025). A lipidic matrix and tannin blend microencapsulated in an escape lysine strategy in sheep improved apparent digestibility indices and total digestible nutrients. This means that the AA delivery platforms are shielded and can significantly change the digestive economy despite performance responses being determined by the base constraint and attained availability (Oliveira *et al*., 2025). Methionine is an essential amino acid, is distinctive due to its structure that contains sulphur (Yu et al., 2025). In the case of methionine, N-acetyl-L-methionine derivative evidence indicated an enhancement of plasma levels of methionine reactions as well as patterns that are attributable to deacetylation preceding circulation, which supports that post-ruminal conversion ability is considered a component of efficacy (Huang *et al*., 2023). The rumen protection is conceptually framed in work on controlled-release nitrogen technology, which tries to view rumen protection as an engineering concept of controlling the location and timing of nitrogen substrates in the gut ecosystem (Fig. 3). In the case of microbiota modulation, this is mechanistically applicable, although the guarded nutrient is not an amino acid (Bezerra *et al*., 2025).

Along with lysine and methionine, functional amino acid supplementation offers supplementary proof that amino acid provision can connect the microbial ecology to aiding ruminant microbiome phenotypes (Gilbreath *et al*., 2021). The supplementation of L-glutamine in Hu lambs fed on a high-concentrate finishing di*et al*tered the rumen fermentation indices and altered the ruminal microbiota. At the same time, ruminal epithelial responses featured decreased transcripts of pro-inflammatory cytokines (e.g., IL-6 and TNF- a) and amplified anti-inflammatory signaling (e.g., IL- 10) along with the variation in tight-junction and barrier-related genes (Wu *et al*., 2025). This paper focused on the rumen epithelium, as opposed to the jejunum or the ileum, but it was research that supported the larger mechanistic assumption that localized AA delivery can alter microbial metabolites and host barrier/ inflammatory mechanisms. This type of interpretation is directly applicable to assessing rumen-protected lysine and methionine products.

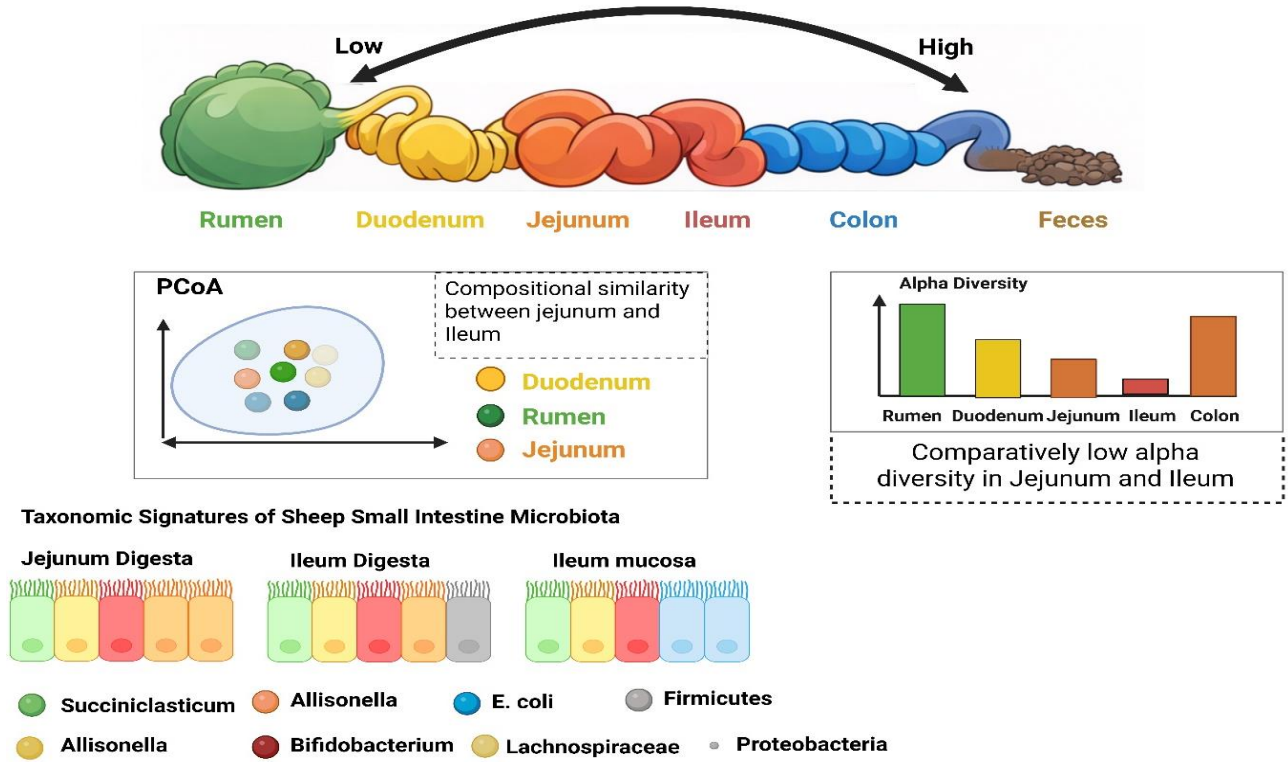


Fig. 3: Baseline Microbial Profiling Across the Sheep Gastrointestinal Tract. Note: PCoA, principal coordinate analysis; E. coli, *Escherichia coli*.

Bioavailability as a confounder in RPAA studies: The essential limitations to comparison between rumen-protected amino acid (RPAA) studies include variations in ruminal stability, intestinal release, and whole-animal bioavailability. Relative bioavailability can be modest even with a product demonstrating rumen protection, plasma response, and other measures of in vivo. "Bypass", therefore, does not always mean bypass at the tissue level, as methionine/lysine is usable (Räisänen *et al*., 2025).

A second constraint is methodological, because bioavailability estimates vary among assessment methods and standardization. Inconsistent verification can distort mechanistic interpretation when studies attribute microbiota or barrier effects to the delivery of post-ruminal amino acid (Estes *et al*., 2022; Du *et al*., 2025). Thus, findings of RPAA must be weighted by the fact that the study either directly confirms bioavailability and release properties (Table 1), and future research must provide bioavailability data as well as segment sample (jejunum/ ileum, digesta vs. mucosa) to enhance the use of causal inference.

Small-Intestinal Microbiota in Sheep: Baseline Characteristics: The evidence from the sheep gastrointestinal tract's baseline flora indicates a strong regional clustering of microbial communities. The microbiome in the rumen is primarily responsible for ruminants' capacity to break down and digest unusable material (Zhou et al., 2024). It also reveals relatively low alpha diversity in the jejunum and ileum compared to other compartments, indicative of physiological screening and high turnover in these locations. In this midgut area, the jejunum and the ileum may be compositionally close. There have been reports of some profiling of tract-wide data, where most locations are considered to be unique communities, except the jejunum and ileum that were not distinctly differentiated in the conditions of their study (Sha *et al*., 2024; Su *et al*., 2025). Early-life Hu sheep are also described to be segment- and age-dependent, in which jejunal diversity can temporarily be high at birth. This strengthens the theory that the developmental phase may overlap with dietary measures in determining outcomes of microbiota observed. Taxonomic descriptions in sheep tract datasets report enrichment of taxa, such as Succiniclasticum, Allisonella, and Lachnospiraceae members, with a comparatively higher presence of Escherichia and Bifidobacterium at small-intestinal sites in those datasets. This shows that Firmicutes are usually found to co-exist with Proteobacteria and Actinobacteriota signatures in small-intestinal communities, depending on the context (Zhang *et al*., 2021a; Li *et al*., 2023; Su *et al*., 2025).

The first interpretive limitation is that mucosa-associated microbiota might vary significantly compared to fecal microbiota. The evidence from ileal mucosa is also characterized by specific clustering and functional signatures in comparison with feces. This favors the use of fecal data as background, and not first-line indicators of jejunal and ileal mechanisms (Derakhshani *et al*., 2016; Juge, 2022). The technical descriptions suggest that mucosa collection includes washing and scraping epithelial surfaces, then sequencing them. This supports that the sampling location and the sampling technique determine what can be said about host-microbe interface biology (Stabel *et al*., 2019; Yan *et al*., 2024; Yao *et al*., 2024). These baselines can be quickly disturbed by the effects of diets. As an example, high-grain feeding of dairy cows has been shown to change mucosa-associated bacteria in the jejunum and ileum, with signs of inflammation and injury of the barrier. This shows that communities of mucosa are capable of adapting to diet composition at the small-intestinal locations (Lai *et al*., 2022; Chen *et al*., 2023).

Table 1: Rumen-protected nutrient platforms are represented in the attached evidence base: protection approach, bioavailability evidence, and dosing context.

Platform class	Example (from attached papers)	Protection/release concept	Bioavailability evidence (reported)	Dose used (reported)	Species/context
Fat-/lipid-coated RP-Lys	Commercial fat-coated lysine product evaluation	Lipid coating intended to resist rumen degradation and release post-ruminally	Relative bioavailability estimated from plasma-response methods; rumen-incubation studies indicate substantial retention of N in coated form (Fagundes, 2021)	Varied by inclusion/mixing context (Fagundes, 2021)	Dairy-cow oriented evaluation
Chemical derivative (RP-Met)	N-acetyl-L-methionine (NALM)	Molecular modification reduces ruminal loss; deacetylation occurs before/within absorption	Increased plasma methionine response; acetylated form not detected in blood, consistent with deacetylation before circulation (Fagundes, 2021)	30 and 60 g/day	Ruminant supplementation study
Wax + tannin microencapsulation (RP-Lys)	Carnauba-wax matrix with tannin blend ("escape lysine")	Hydrophobic wax matrix + tannin complexation to reduce ruminal degradation and permit intestinal release	Improved apparent digestibility indices and TDN; performance response depends on baseline limitation and realized availability (Oliveira *et al*., 2025)	3% and 6% of diet (Oliveira *et al*., 2025)	Sheep
Post-ruminal nutrient delivery analog	Rumen-protected glucose (RPG)	Protected nutrient used as proof-of-principle for post-ruminal delivery	Increased villus height and VH/CD; altered ileal SCFA profile; reduced LPS and TNF-α; increased ileal barrier gene expression (Claudin-1); shifted ileal microbial community (e.g., ↑Bifidobacterium/Aeriscardovia) (Gao *et al*., 2025)	0.08% of diet; 90-day trial	Tibetan sheep (male lambs)
Controlled-release N (conceptual parallel)	Slow-release urea technology (review)	Coatings regulate ruminal N release kinetics to improve synchronization and N efficiency	Conceptual synthesis emphasizing variability across products and dependence on coating and release kinetics (Bezerra *et al*., 2025)	Product-dependent	Ruminants (review)
Bioavailability methodology synthesis	Review/thesis on RPAA bioavailability methods	Highlights methodological sensitivity of BA estimates and the need for standardization	BA estimates vary widely across products; instability/variability can distort mechanistic interpretation (Sandoval, 2015)	—	Methodological synthesis

Note: RP, rumen-protected; RP-Lys, rumen-protected lysine; RP-Met, rumen-protected methionine; NALM, N-acetyl-L-methionine; RPG, rumen-protected glucose; RPAA, rumen-protected amino acids; BA, bioavailability; TDN, total digestible nutrients; VH/CD, villus height to crypt depth ratio; SCFA, short-chain fatty acids; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; N, nitrogen.

Experiments using Lys: Met ratios as a dietary manipulation show that a diet-dependent remodeling of the jejunal ecology occurs, though these effects are not consistent across endpoints and study designs. A low-protein diet with an optimal Lys: Met ratio in Tibetan sheep corresponded to better jejunal morphology and antioxidant status and lower inflammatory markers. It was also linked with augmented wealth and taxa alterations, including Romboutsia and Lachnospiraceae related clans. Integration of multi-omics connected these differences within communities to specific metabolite phenotypes, suggesting a combined microbiome-metabolome approach to a single set of isolated taxonomic changes (Zhang *et al*., 2025). However, cross-study comparability remains constrained because responses likely depend on baseline crude-protein levels, the degree of amino-acid limitation, and whether outcomes are measured in digesta versus mucosa. These confounders are rarely standardized, which can make "optimal" ratios appear study-specific. A practical implication is that Lys: Met effects should be interpreted as conditional on the low-protein context and validated using consistent segment sampling and barrier/immune readouts, rather than relying on microbiota alone.

Complementary multi-omics evidence further supports the link between limiting amino acid balance, jejunal ecology, and barrier readouts. In lambs whose diets were low in proteins that had graded lysine: methionine ratios, a low Lys: Met ratio enhanced the richness of jejunal bacteria and upregulated genes associated with barriers (e.g., Occludin and Muc2) and downregulated genes associated with pro-inflammatory cytokines (e.g., IL-6 and TNF-α) in jejunal tissue. Metabolomic data indicated that the covariation between these morphological and immune indices and bile-acid-related metabolites and other small molecules. This confirms the idea that AA-induced changes in microbiota could be a measurable phenotype of epithelial barriers that cannot be explained by a shift in taxa alone.

Multi-compartment evidence with rumen-protected sulfur-containing amino acid (AA) supplementation in Tibetan sheep has shown not only coordinated rumen and jejunal microbial changes but also improved carcass traits. This suggests that protected delivery of sulfur-AA can reconstruct the community (comprising both compartments) and connect microbial characteristics to sites of production (Liu *et al*., 2024). The increases in taxa are reported and are frequently discussed in the context of fermenting carbohydrates and volatile fatty acid generation, such as Prevotella, Butyrivibrio, Lachnospiraceae, and Ruminococcaceae, and decreasing Turicibacter, which further supports the opinion that the protective AA supplementation can manipulate the microbial assembly. The changes are likely associated with fermentation products. Simultaneously, evidence from the colon metagenome in lambs indicated that low-protein dietary intervention selects a community with a higher proportion of *Escherichia coli*. In the same context, combined rumen-protected methionine and lysine supplementation enriched *Akkermansia muciniphila* and increased the predicted potential for volatile fatty acid and amino acid biosynthesis. This indicates that protected AA supplementation can restructure distal gut ecology under low-protein challenge (Luo *et al*., 2025).

Hu sheep evidence strengthens the relevance to the target species and intestinal segments. Rumen-protected glucose in Hu sheep altered ileal microbial features and volatile fatty acid readouts and was associated with the regulation of inflammatory and tight-junction gene expression in the ileum, providing segment-level evidence that post-ruminal nutrient delivery can remodel ileal ecological and barrier pathways in this species. A Hu sheep ileal mucosa and metabolome dataset further reports that ileal mucosal microbiota and metabolites form a connected network with diet-dependent correlations between disrupted OTUs and differential metabolites, reinforcing that ileal ecology is intertwined with metabolic coupling at the mucosal interface. At the whole gastrointestinal tract scale in Hu sheep, switching from alfalfa to wheat straw under isocaloric and isonitrogenous conditions produced the most pronounced microbial response in the rumen and highlighted strong compartmentalization across the tract, while also indicating compartment-specific restructuring of microbial networks and predicted functions after diet shift. These Hu sheep find position diet-driven substrate changes as tract-wide drivers whose downstream consequences for small-intestinal niches must be interpreted within a compartmentalized system rather than assumed from rumen changes alone (Fig. 4) (Li *et al*., 2023).

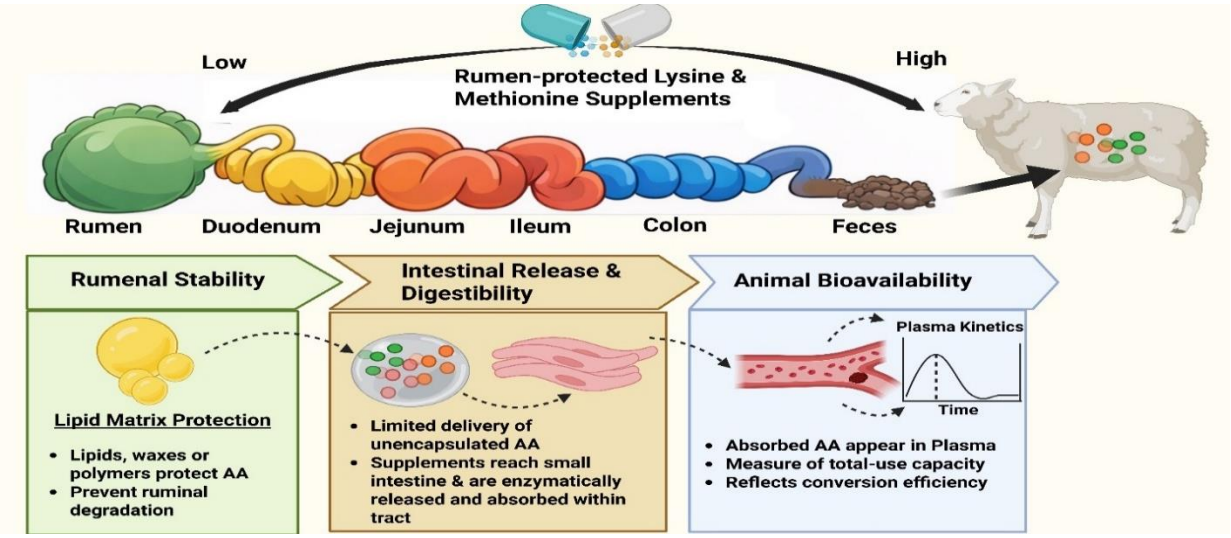


Fig. 4: Rumen-Protected Lysine & Methionine: Dynamics of Supplementation. Note: AA, amino acid.

Key segment-level intervention studies linking post-ruminal amino acid supply to jejunal/ileal microbiota, metabolites, and host outcomes are summarized in Table 2. A translational pathway map summarizing the proposed diet–substrate–microbiota–barrier/immune mechanisms are shown.

Mechanistic Links: Microbiota, Barrier Function, and Host Metabolism: A mechanistic chain consistent across the evidence base links dietary protein degradability and protected AA delivery to changes in the chemical forms of nitrogen and associated metabolites entering the small intestine. The alterations in epithelial transport, barrier signaling, and remodeling microbial selection forces act at the jejunal and ileal locations. RUP:RDP manipulation in lambs was associated with digestibility and nitrogen-use changes and ruminal fermentation patterns, such as altered propionate dynamics, supporting that degradability affects fermentation product landscapes and nitrogen partitioning that propagate downstream (Valizadeh *et al*., 2021). The predicted functional profiles in the Hu sheep roughage-transition context changed by switching from alfalfa to wheat straw, and microbial abundance networks became more complicated in compartments. This shows that the diet has the ability to rewire taxa, as well as the structure of the microbial interaction and the inferred metabolic capacities, which are likely to change metabolite fluxes into distal segments (Li *et al*., 2023).

Tryptophan-Derived Metabolites and Aromatic Amino Acid Signaling (AhR/mTOR Axis): Aromatic amino metabolites and tryptophan-derived signaling (AhR/mTOR axis). Along with nitrogen and short-chain fatty acid metabolism, aromatic amino acid metabolism, particularly tryptophan metabolism, is a mechanistic intermediary between diet, microbiota, and mucosal immunity. Microbial metabolites of tryptophan can activate epi- and innate immune receptors, e.g., aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR). These metabolites influence the proliferation of epithelial cells, integrity of the barrier, antimicrobial defense, and the inflammatory condition (Fig. 3) (Gargaro *et al*., 2021).

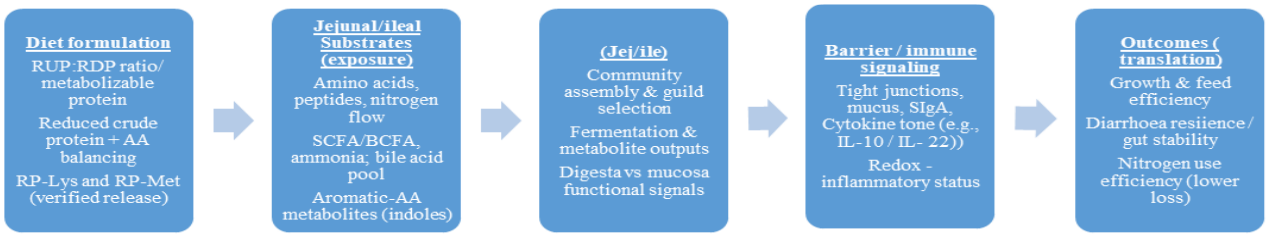
Primary experimental data have shown that tryptophan metabolites (indole-3-ethanol and indole-3-pyruvate) of microbes prevent colitis-related permeability and maintain epithelial barrier activity. They also stabilize the apical junction complex and/or regulate cytoskeletal machinery, ezrin and nonmuscle myosin IIA, in an AhR-dependent way (Scott *et al*., 2020; Jia *et al*., 2022; Shi *et al*., 2025; Donglin *et al*., 2026).

These pathways are essential in understanding the effects of RUP and rumen-protected lysine/methionine interventions since alterations in the supply of post-ruminal amino acids have the capacity to modify how the microbes have access to the aromatic feeds. They can also change the ratio between indole synthesis and host tryptophan degradation (e.g., the kynurenine pathway), and such downstream effects on epithelial barrier and innate immune signals (Ma Ning and Ma Xi, 2019).

Table 2: Segment-level and gut-axis evidence linking protein/AA strategies to microbiota, metabolites, and host outcomes in sheep (selected examples).

Study (species/context)	Intervention	Sampling site(s)	Key microbiota /omics signals	Key outcomes (host/production)
(Ji *et al*., 2024) (Tibetan sheep)	Low-protein diet with optimized Lys:Met balance	Jejunum (microbiome + metabolome + tissue indices)	↑ richness and shifts toward *Romboutsia*, *Ruminococcus* groups, and Lachnospiraceae; metabolite shifts involving β-alanine, pantothenate/CoA and cysteine-related pathways	Improved villus morphology and antioxidant status; reduced inflammatory markers in jejunal tissue
(Wu *et al*., 2025) (pregnant sheep)	Undernutrition (50% of energy and protein requirements)	Jejunum and ileum (microbiota; fermentation and epithelial indices)	Altered community structure (including Lactobacillaceae and Prevotellaceae signals); altered VFAs and microbial protein	Downregulation of epithelial homeostasis pathways, supporting the sensitivity of small-intestinal niches to nutrient scarcity
(Zhang *et al*., 2024) (sheep)	Low-protein diets with graded soluble protein (SP) levels	Gut-axis readouts (intestinal N absorption; fecal microbiota and metabolome)	Microbiota and metabolome reprogramming under SP manipulation in low-CP diets	Improved energy utilization and intestinal N absorption; associations consistent with reduced fecal reactive nitrogen emissions
(Pelegrin-Valls *et al*., 2020) (light lambs)	Modest dietary crude-protein reduction	Rumen and ileal tissue; systemic inflammation markers	No major disruption reported in inflammation-related gene expression with CP reduction	Improved feed conversion without adverse rumen/ileal/systemic inflammatory gene-expression responses
(Cai *et al*., 2025) (Hu lambs)	Dietary metabolizable protein levels (graded MP:ME)	Rumen microbiota; plasma amino-acid profile; performance	MP level influenced rumen bacterial community and plasma AA profile	Intermediate MP supply improved average daily gain and supported improved nitrogen-use outcomes
(Zhang *et al*., 2021) (Hu sheep)	Rumen-protected glucose (post-ruminal nutrient delivery as mechanistic analog)	Ileum (microbiota, VFAs; barrier/inflammation gene expression)	Altered ileal microbial features and VFAs	Modulation of tight-junction and inflammatory gene expression in ileal tissue

Note: AA, amino acid; Lys, lysine; Met, methionine; Lys: Met, lysine-to-methionine ratio; VFAs, volatile fatty acids; SP, soluble protein; CP, crude protein; MP, metabolizable protein; ME, metabolizable energy; MP:ME, metabolizable protein-to-metabolizable energy ratio; N, nitrogen; CoA, coenzyme A.



Another microbial metabolite axis, which may interact with aromatic AA signaling and barrier immunity, is the process of bile acid transformation. In a goat model of small intestinal inflammatory injury, microbial communities in the ileum were disrupted and accompanied by reduced levels of secondary bile acids and a change in Th17/Treg balance, with pathway analysis indicating involvement of MAPK/PPAR signaling (Fig. 5) (Wang *et al*., 2024). Despite the species and disease differences, these data provide support for the possibility of diet-induced changes in jejunal/ileal microbial metabolism, including bile acid pools as evidence of altered metabolites in the studies of AA-balancing, having an impact on immune and barrier pathways applicable in intestinal resilience in sheep.

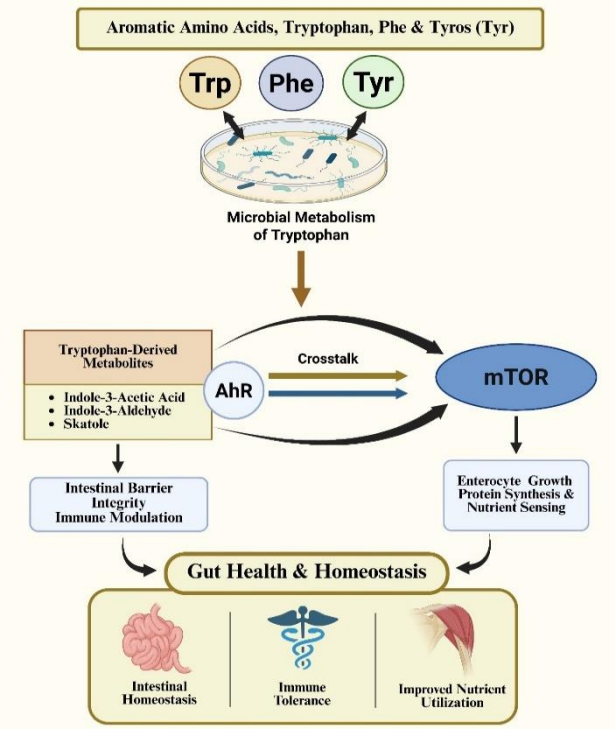


Fig. 5: Microbiota–Aromatic Amino Acid Interactions Regulating AhR–mTOR Crosstalk in Intestinal Homeostasis. Note: Trp, tryptophan; Phe, phenylalanine; Tyr, tyrosine; AhR, aryl hydrocarbon receptor; mTOR, mammalian target of rapamycin.

The AA balance of low-protein diets at the jejunal level provided direct argument that substrate manipulation can lock the metabolite signatures to changes in microbes and phenotypes of host tissues. A low-protein diet optimized with lysine-to-methionine levels is linked to a unique jejunal metabolome, such as beta-alanine, pantothenate/CoA-related products, and cysteine-related products. It is also related to enhanced villus morphology, antioxidant conditions, and decreased inflammatory indicators. These data indicate a mechanistic theory whereby the change in AA supply reconfigures the jejunal microbial metabolism that has downstream outcomes on epithelial physiology. Under protected sulfur-AA supplementation, reported correlations between rumen and jejunal bacteria and meat metabolite characteristics suggest that assemblies of microbes can control metabolite products, which follow production characteristics. This offers a connection between microbial signaling, fermentation chemistry, and host phenotypes indirectly. Evidence of the distal gut under low-protein challenge also shows that rumen-protected methionine and lysine have the potential to enrich taxa and that the potential of volatile fatty acid and amino acid biosynthesis is predictable in the colon. This facilitates a microbial-metabolic route such that the supplementation affects the products of fermentation and nitrogen management outside of the rumen. The existence of ileal mucosa in Hu sheep demonstrates the mechanisms of host and microbe interaction wherein the microbiota and metabolites engage in interrelated networks with diet-specific relationships. This supports the idea that the mucosal communities are involved in host-microbe metabolic coupling and not as detached luminal communities. Segment-level causal plausibility for nutrient-delivery effects on the ileal barrier and inflammatory pathways is illustrated by rumen-protected glucose in Hu sheep, which was associated with ileal microbial changes, altered volatile fatty acid readouts, and regulation of inflammatory and tight-junction-related gene expression in the ileum. More broadly, ruminant nutrigenomics framing emphasizes that diet can reshape metabolite flux and barrier biology, including the role of short-chain fatty acids in epithelial barrier integration. It also emphasizes the need for multi-layered molecular approaches to disentangle diet-induced effects from developmental noise. This aligns with the multi-omics designs used in jejunal microbiome–metabolome studies under AA balancing (Abdelrahman *et al*., 2022).

Finally, causal microbe–host coupling evidence from early-weaned lambs indicates that direct microbiota manipulation via fecal microbiota transplantation increased taxa, such as Bifidobacterium and Collinsella, and was negatively correlated with diarrhoea and inflammation indices. It has also been reported that relationships exist among microbial taxa, inflammatory markers (including IL-1β and TNF-α), and host responses, including changes in transport and inflammatory gene expression. These findings reinforce that microbiota restructuring can track barrier and immune signaling outcomes relevant to small-intestinal health (Fig. 6) (Guo *et al*., 2024). This causal arm strengthens the plausibility that dietary AA delivery strategies could act as non-antibiotic nutritional levers to influence host–microbe systems during stress windows when the jejunal and ileal communities are plastic.

Implications for Growth Performance, Immunity, and Intestinal Health in Hu Sheep: Other studies have demonstrated the possibility of reducing the amount of crude protein in feeds without compromising intestinal immune outcomes. In light lambs, a modest reduction in dietary crude protein improved feed conversion without adversely affecting rumen, ileal, or systemic inflammatory gene expression markers, suggesting that CP reduction can be compatible with gut health when the nutrient supply is adequate (Zhang *et al*., 2020). In Hu lambs, varying dietary metabolizable protein levels altered growth and plasma amino acid profiles, and an intermediate MP supply improved average daily gain while shifting ruminal bacterial community structure, reinforcing that MP targeting is an actionable lever for productivity and nitrogen utilization.

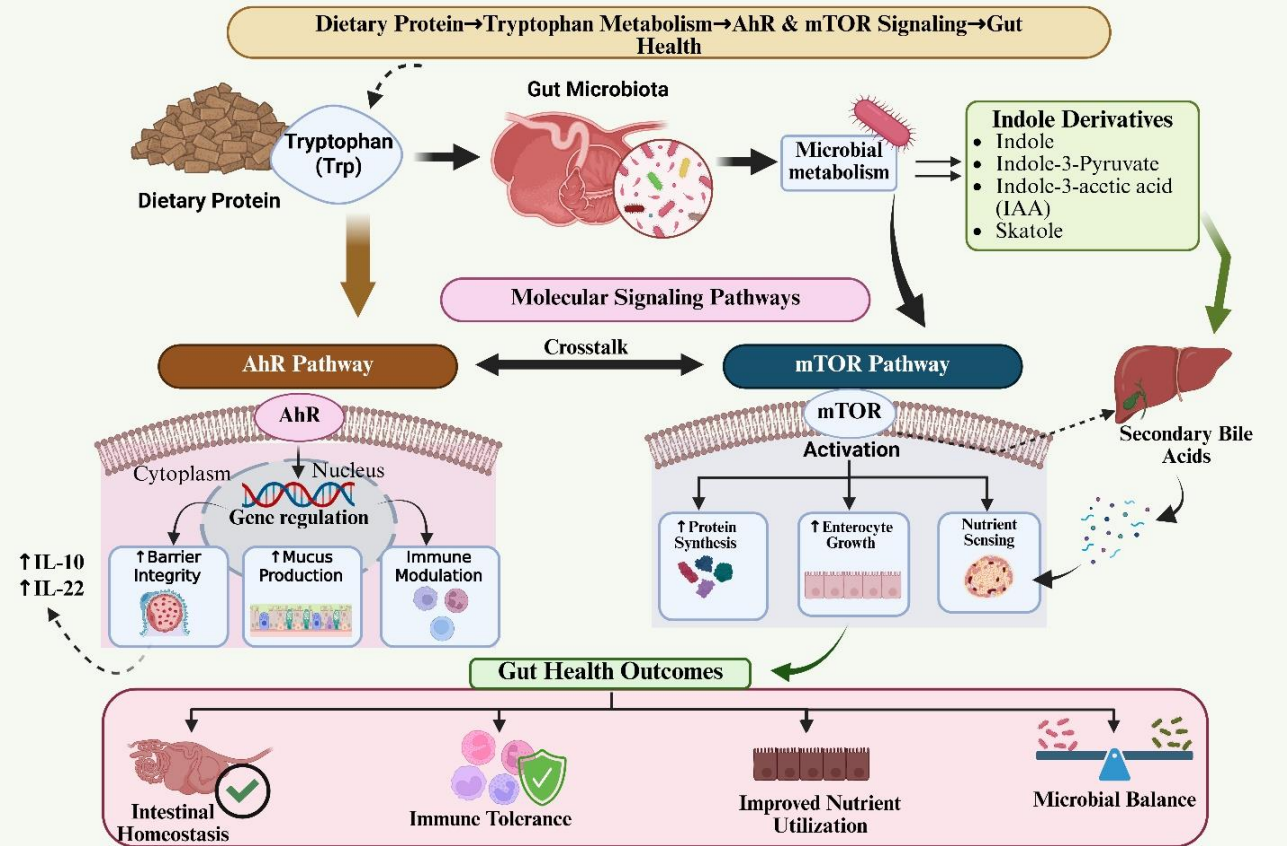


Fig. 6: Aromatic Amino-Metabolites and Tryptophan- Derived Signaling (AhR/mTOR Axis). Note: Trp, tryptophan; AhR, aryl hydrocarbon receptor; mTOR, mammalian target of rapamycin; IAA, indole-3-acetic acid; IL-10, interleukin-10; IL-22, interleukin-22.

Evidence specific to Hu lambs also supports metabolizable protein (MP) as an actionable lever for combining performance and environmental outcomes. In a controlled feeding trial in 2–4-month-old female Hu lambs, a moderate dietary MP level (8.66% of DM) improved average daily gain and increased nitrogen utilization efficiency relative to a lower MP diet. It was also accompanied by shifts in rumen bacterial community structure, including increased abundance of *Christensenellaceae_R-7_group* (Cai *et al*., 2025). Although this study quantified rumen microbiota rather than jejunal or ileal microbiota, it supports the overarching hypothesis that optimizing post-ruminal protein supply can refine nitrogen allocation while remodeling the microbial community in Hu sheep production systems.

In Hu sheep, intestinal health concerns are heightened by the on-farm relevance of diarrhoea. Hu lamb diarrhoea was correlated with reduced intestinal bacterial diversity, reduced Bacteroidetes, a shift toward more aerobic or stress-resistant bacterial types, and lower serum total cholesterol, IgG, and IgM. These findings link microbial disturbance with broader biochemical and immunological signals. This relationship suggests that nutritional interventions that modify intestinal ecology may lead to clinically significant improvements in growth metrics in Hu lamb production. The plausibility that diet can influence ileal barrier function and inflammatory pathways in Hu sheep is supported by evidence from rumen-protected glucose supplementation. This evidence shows changes in ileal microbiota, altered volatile fatty acid profiles, and regulation of inflammatory and tight-junction gene expression in the ileum. An ileal mucosa microbiota–metabolome network in Hu sheep further supports that ileal communities are coupled to metabolite patterns under dietary modulation, implying that nutritional shifts can reconfigure host–microbe metabolic interfaces at the mucosal level.

Evidence from functional amino acid interventions also supports the concept that post-ruminal nutrient delivery can reshape downstream metabolite axes relevant to gut resilience. In late-gestation ewes, rumen-protected glutamine supplementation increased offspring serum glutamine levels. This was associated with enrichment of phenylalanine/tyrosine/tryptophan biosynthesis and reduced kynurenine-pathway metabolites (e.g., kynurenine and anthranilic acid). This pattern is consistent with a shift away from tryptophan degradation pathways, which are often linked to pro-inflammatory responses. Although this work was not performed in Hu sheep and did not include rumen sampling, it illustrates how targeted post-ruminal amino acid delivery can modulate aromatic amino acid metabolism and immune-biochemical phenotypes beyond growth metrics (Nie *et al*., 2025).

Both the nutritional and microbial intervention datasets reinforced the implications for immune and barrier integrity. Hydrolyzable tannin supplementation improved small-intestinal antioxidant capacity and barrier markers at an effective dose but suggested permeability stress at a higher dose. This emphasizes that intestinal resilience responses may be dose-dependent and that “more” supplementation does not necessarily improve barrier outcomes. Faecal microbiota transplantation in early-weaned lambs demonstrated that microbiota restructuring can coincide with reduced diarrhoea/inflammation indices

and altered host inflammatory and transport gene expression, providing evidence that intestinal health endpoints are microbiota-sensitive and nutritionally targetable during stress windows. In this context, dietary AA delivery strategies that shift luminal substrates and epithelial signaling are positioned as plausible non-antibiotic approaches to support gut resilience, provided that interventions are anchored to demonstrated post-ruminal availability rather than protection labels.

Research Gaps and Future Directions: A central gap is that AA requirement concepts and "ideal protein" templates may be context-dependent across species, physiological states, diets, and environments. Extending fixed AA-ratio paradigms across diverse production settings may underestimate the influence of diet composition, genetics, and downstream metabolic handling in small ruminants (Wu and Li, 2022). Quantitative benchmarks exist for proximal duodenal AA patterns in growing sheep, offering a physiologic reference for interpreting what intestinal sites are "trying to see" and why protected lysine and methionine might meaningfully shift substrate supply. However, these benchmarks should be treated as references rather than absolutes when extended to varying objectives and diets (Wang and Lu, 2002). Production objectives can also alter limiting-AA priorities; for example, methionine is a key limiting AA for wool growth biology in wool-producing sheep, suggesting that segment-level microbiota effects of protected methionine may differ depending on whether growth or wool is the dominant constraint (Nolte, 2006).

An additional gap is that most AA-focused nutritional studies under-measure functional intermediary pathways that connect amino acid supply to mucosal phenotypes. Segment-specific profiling of tryptophan-derived indoles, kynurenines, and bile-acid pools, combined with epithelial barrier assessments (including tight-junction genes/proteins and mucus markers), would enable explicit testing of AhR/PXR-linked barrier mechanisms. It would also enable testing of metabolite–immune interactions, which are increasingly recognized as central to gut resilience. In practice, integrating these measurements with demonstrated post-ruminal release/bioavailability and compartment-specific sampling (digesta vs. mucosa) would reduce the risk of attributing microbiota effects to 'protected amino acids' when intestinal exposure is not confirmed.

A second gap is segment-specific causality linking dietary AA delivery to jejunal and ileal metabolite landscapes, barrier readouts, and immune responses with appropriate controls. Hu lamb diarrhoea evidence provides disease-linked microbiota and immune-biochemical signatures, but controlled mechanistic pathways connecting AA delivery to these outcomes remain thin. Although fecal microbiota transplantation demonstrates that microbial restructuring can influence inflammation and diarrhoea-related endpoints in early-weaned lambs, comparable causal designs are still needed to bridge the association with the mechanism. These designs should integrate dietary AA delivery, jejunal/ileal sampling, metabolites, and host gene expression outputs. Host-side mechanisms are repeatedly described as requiring multi-omics approaches in ruminant nutrigenomics, emphasizing that diet–microbiota interactions should be resolved through integrated molecular and metabolite analyses rather than solely by taxonomic descriptions.

A third gap is methodological, about the sampling site and inference. Evidence that ileal mucosa-associated microbiota differs from fecal communities and that ileal mucosal work remains limited implies that future studies should prioritize segment-specific mucosal sampling in Hu sheep rather than extrapolating from fecal profiles. Tract-wide Hu sheep evidence also emphasizes compartmentalization, showing that location effects can dominate diet effects and that dietary shifts can rewire network structure differently across compartments, suggesting that mechanistic conclusions about jejunum and ileum require direct segment sampling rather than rumen-derived inference (Li *et al*., 2023). This is especially relevant when protected AA strategies are assessed, because rumen protection and post-ruminal release can vary, and bioavailability may be modest or unstable across products, implying that downstream intestinal effects may be attenuated unless availability is confirmed in the target context.

Finally, the sustainability implications of feeding interventions remain inadequately quantified, highlighting the need for more comprehensive evaluations. System-level literature frames nitrogen-use efficiency as being influenced by the forage base and agroecological management, and amino acid adequacy at lower crude protein levels is conceptually attractive for tightening nitrogen partitioning. Feeding interventions should be evaluated based on measured nitrogen retention and excretion rather than assumed sustainability gains. Given that RUP:RDP manipulation can shift nitrogen utilization indicators in lamb trials, integrating nitrogen partitioning endpoints with jejunal/ileal microbiota and barrier readouts would provide stronger translational evidence for sheep production systems.

Conclusions: Evidence from ruminant protein evaluations, sheep intervention trials, and small-intestinal microbiota studies supports a coherent framework. In this framework, dietary protein degradability and rumen-protected lysine and methionine alter the quantity and form of nitrogenous substrates entering the jejunal and ileal segments, with plausible downstream effects on microbial assembly, metabolite profiles, and host barrier and immune signaling. Manipulating RUP:RDP ratios improves lamb growth performance and nitrogen-use indicators in controlled trials, indicating that post-ruminal AA supply is an actionable lever. Gut physiology imposes strong splanchnic extraction that separates luminal substrate delivery from systemic AA availability in mechanistic interpretation. Rumen-protected AA strategies are mechanistically credible nutritional modulators of small-intestinal niches. However, efficacy depends on demonstrable post-ruminal release and bioavailability, which can vary widely across products and methodologies. In Hu sheep, segment-level evidence that post-ruminal nutrient delivery reshapes ileal microbial and barrier-related gene expression, alongside ileal mucosa microbiota–metabolome coupling, supports a host–microbe interaction model relevant to intestinal health. Given that Hu lamb diarrhoea is associated with reduced

intestinal bacterial diversity and altered immunological profiles, advancing RUP and protected lysine/methionine strategies as gut-resilience tools will require segment-specific, multi-omics trials. These trials should link dietary AA delivery to jejunal/ileal metabolites, barrier function, immunity, and nitrogen partitioning outcomes within the compartmentalized ruminant gut system.

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Abbreviations:

- AA Amino Acid
- CP Crude Protein
- MP Metabolizable Protein
- ME Metabolizable Energy
- DM Dry Matter
- RUP Rumen-Undegradable Protein
- RDP Rumen-Degradable Protein
- RP Rumen-Protected
- RPAA Rumen-Protected Amino Acids
- RPG Rumen-Protected Glucose
- RP-Lys Rumen-Protected Lysine
- RP-Met Rumen-Protected Methionine
- Lys Lysine
- Met Methionine
- Lys: Met Lysine to Methionine Ratio
- NALM N-Acetyl-L-Methionine
- SP Soluble Protein
- TDN Total Digestible Nutrients
- BA Bioavailability
- VFAs Volatile Fatty Acids IL-6-Interleukin-6
- IL-10 Interleukin-10
- IL-1β Interleukin-1 Beta
- TNF-α Tumor Necrosis Factor Alpha
- IgG Immunoglobulin G
- IgM Immunoglobulin M
- LPS Lipopolysaccharide
- VH/CD Villus Height to Crypt Depth Ratio
- Muc2 Mucin 2
- OTUs Operational Taxonomic Units
- AhR Aryl Hydrocarbon Receptor
- PXR Pregnane X Receptor
- mTOR Mammalian Target of Rapamycin
- MAPK Mitogen-Activated Protein Kinase
- PPAR Peroxisome Proliferator-Activated Receptor
- AMPK AMP-Activated Protein Kinase
- Th17 T Helper 17 Cells
- Treg Regulatory T Cells
- CoA Coenzyme A
- SCFA Short-Chain Fatty Acid(s)
- β-alanine Beta-Alanine
- MP: ME Metabolizable Protein to Metabolizable Energy Ratio in vivo within a living organism

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