Nutritional Strategies to Mitigate Enteric Methane Emissions in Dairy Buffaloes

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Abstract: Water buffalo (Bubalus bubalis) is an important multi-purpose ruminant species providing animal-based food and supporting rural communities' economic sustainability, especially in Asia. However, the specialized microbial consortium responsible for fiber degradation within the rumen also contributes to environmental degradation through enteric methane (CH4) generation, making methanogenesis reduction a pivotal challenge for the sector's sustainable development. As CH4 results from ruminal microbiota metabolism, nutritional interventions are regarded as having the greatest mitigation potential, although most studies have focused on cattle, and buffalo-specific data remain scarce. The literature offers a broad range of effective strategies, including modifications to the forage-to-concentrate ratio and dietary lipids and phytochemicals, with mitigation gains ranging from 10 to 80%. These strategies can reduce methanogenesis through several mechanisms, including direct inhibition of methanogenic archaea and protozoa, shifting fermentation pathways, or diverting hydrogen toward alternative sinks. However, *in vivo* studies in buffalo remain scarce, underscoring the need for future research that considers all CH4-relevant parameters within a holistic framework. The available literature remains limited and fragmented, underscoring the need for further research to understand species-specific responses and develop effective mitigation strategies under practical conditions. Therefore, this review comprehensively summarises current knowledge of nutritional interventions that have been shown to reduce CH4 production in buffalo without adverse effects on welfare or productivity. Additionally, given their influence on CH4 production and implications for species-specific responses, the main differences in digestive morphology, physiology, and rumen microbial ecology between buffalo and cattle are discussed, providing a comparative perspective to evaluate whether mitigation approaches developed for bovines can be effectively applied to buffaloes.

Keywords: Buffalo nutrition, sustainability, methane, rumen, phytochemicals, feeds.

1. INTRODUCTION

The buffalo species (*Bubalus bubalis*) is an integral part of livestock production, particularly as a source of income and animal protein in the form of milk and, to a lesser extent, meat [1-3]. Moreover, in tropical areas, the buffalo serves multiple roles within integrated crop-livestock farming systems, acting as a draft animal, providing sustenance to rural communities, and contributing to nutrient cycling through grazing and manure excretion, thereby enhancing soil carbon content [4]. Although more than 90% of the population is reared in Asia [2], buffalo rearing is also an important contributor to local economies in many European and South American countries [5-9]. Southern Italy has historically been devoted to buffalo rearing, which supports local employment and the economy [5, 6, 9]. Furthermore, growing buffalo milk demand has led to the conversion of buffalo farming from extensive to intensive systems, with the adoption of dairy-sector practices [5, 6, 10]. Notably, beyond the traditional value, consumers appreciate the sensory properties and favorable amino acid and fatty acid profiles of buffalo-derived products, which align with healthy diet principles [6, 7, 11]. However, intensification raised concerns about the high water footprint of certain ingredients in lactating buffaloes' diets (e.g., corn silage), which endangers economic and environmental sustainability [12, 13]. Furthermore, energy and nutrient-dense diets negatively impact animal health and productivity [14]. Rather, strengthening rural areas' agricultural production preserves their cultural heritage and may foster sustainable development of the buffalo sector [6, 7, 15].

Nevertheless, ruminant rearing contributes to anthropogenic greenhouse gas (GHG) emissions, including methane (CH_4) and nitrous oxide (N_2O) from enteric fermentation, manure management, and feed production. These gases have global warming potentials approximately 25 and 298 times higher than CO_2, respectively [16-18]. In particular, CH4 originates inside the rumen as an inevitable by-product of fiber

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degradation by ruminal microflora and represents the main GHG emitted along the ruminant production chain [17]. Although on a smaller scale than dairy cows, and despite being considered a lower CH_4-emitter based on dry matter intake (DMI) [19], the buffalo sector still contributes to environmental GHG release [6, 15, 17]. Moreover, due to lower productivity, the environmental footprint of 1 kg of normalized buffalo milk has been reported to be several-fold higher than that of cow's milk [17, 20]. Additionally, buffalo emission factors have been found to be significantly higher than those calculated using existing equations and reported to vary seasonally [21], although contrasting patterns have been documented [22]. Recognizing these differences is essential for designing tailored mitigation strategies to promote the buffalo sector's sustainability. Therefore, to continue satisfying the increased demand for animal-based foods without further contributing to environmental degradation, the ruminant supply chain is called to enhance its sustainability [23, 24]. For example, sustainable management practices in which unproductive buffalo heifers are reared on pastures have been identified as a way to reduce GHG emissions with no delay in animal growth [25, 26]. Moreover, recent advances in molecular techniques have improved understanding of the rumen microbial ecosystem, its composition, and its role in fiber fermentation, volatile fatty acid (VFA) production, microbial protein (MP) production, and CH_4 production [4, 27-30]. For instance, phylogenetic marker-based techniques overcome the limitations of traditional culture methods and have become valuable tools for identifying and quantifying the largely uncultivable microbial fraction [30, 31]. Such deeper knowledge about the underlying relationships among ruminants, diet, and the CH_4-producing community is essential for designing targeted interventions to sustainably enhance ruminant nutrition and productivity [29, 32]. Despite the significant mitigation potential of some chemical inhibitors, they are associated with social or health concerns, and considerable efforts are underway to investigate effective alternatives [33]. Under this paradigm, preferred enteric CH_4 emissions' mitigation strategies may be broadly categorized in two groups: i) ruminal ecosystem modulation through dietary manipulation or feed additives and ii) animal selection [24, 33-36].

Nonetheless, CH_4 represents an inevitable by-product of fiber fermentation [17, 37]. Thus, since ruminal microbiota's composition and activity are strongly influenced by diet [38], nutritional strategies intended to modulate the ruminal ecosystem through dietary manipulation or feed additives are regarded as promising approaches to reduce methanogenesis while maintaining animal productivity [24, 34-37]. However, most research on nutritional CH4 mitigation strategies has focused on cattle and small ruminants [24, 31, 33, 36, 39]. Indeed, despite contributing 12% of total enteric CH4 emissions, in a review of more than 400 peer-reviewed *in vivo* studies, buffalo accounted for only approximately 5% of all studies globally, with the first *in vivo* studies first appearing in the mid-2000s onward [39].

Therefore, the lack of a review of nutritional approaches proven effective in reducing enteric CH_4 emissions, specifically focused on the buffalo species, prompted the present paper. Still, owing to the lack of buffalo-specific data, comparisons with bovine studies will be made, emphasizing core differences in feeding behavior, digestive anatomy and physiology, and ruminal microbiota composition, with implications for methanogenesis. Thus, the aim has been to provide a comprehensive overview of valid nutritional strategies, detailing practical information on feed components, effective inclusion levels, quantitative effects, and mechanisms of action, intended for both buffalo farmers and scientists.

2. REVIEW CRITERIA

The present manuscript fills a notable literature gap, offering a narrative review of nutritional strategies to reduce enteric CH_4 emissions, focusing solely on the buffalo species through a holistic lens. In addition, it draws on relevant comparative studies on their physiology and rumen peculiarities regarding bovines, for the dissertation's sake. In fact, their main distinctive structural, physiological, and microbiota-related traits, including enlarged gastrointestinal capacity and greater aptitude for roughages, stronger propionate pathway, and low methanogen presence, which might collectively induce methanogenetic differences compared to cattle, were highlighted, maintaining a rigorous comparative lens against cattle data and explicitly contextualizing buffalo-specific data. In particular, this has been made as no single study has tested the same CH4-mitigating nutritional strategy as a controlled treatment in both cow and buffalo within the same experimental design can be retrieved from the database.

A methodical literature search was conducted between February 2026 and May 2026 to identify a core compendium of relevant studies on nutritional strategies for mitigating enteric methane (CH_4) emissions in buffaloes and to provide scientific support for the present dissertation. The search encompassed the period from the early 2000s to the present 2026, across major electronic databases: PubMed, Web of Science, Scopus, and Google Scholar. The lower limit for publication was set at 2000, as investigations into enteric CH_4 mitigation in buffaloes were negligible prior to this date, with the earliest *in vivo* buffalo CH_4 studies in indexed journals appearing from the mid-2000s onward (Della Rosa *et al*., 2022). Following, an example of employed search string(s), designed to capture species-specific data and mitigation outcomes, as no paper investigating both species at once exists: (“Buffalo“ OR "*Bubalus bubalis*" OR "Cattle" OR "Dairy cow") AND ("enteric CH_4" OR "CH_4 emission" OR “methanogenesis mitigation“) AND (“nutritional strategy" OR “forage” OR “lipid OR "feed additive" OR “phytogenic” OR “probiotic”).

Studies were considered eligible if they met inclusion-specific criteria: i) peer-reviewed original research and review articles, ii) published within the selected timeframe in indexed journals, iii) full text preferably available in English, or alternatively in Italian or Spanish, as these were the languages known by the authors, iv) relevant to the topic; v) measurable outcome. Included studies involve trials on bovines or bubaline animals of any breed, sex, age, or physiological stage (e.g., calves, growing, lactating), or multi-species comparisons (e.g., cattle/dairy cow vs. buffalo), emphasizing buffalo-specific data and reporting main differences separately. To be deemed relevant, studies had to investigate buffalo’s characteristic features or evaluate any dietary manipulation strategy (e.g., feed additive, forage manipulation, concentrate inclusion, dietary lipid, plant secondary metabolite) administered with the aim of lowering enteric CH_4 production. Valid outcomes required at least one direct measurement of enteric CH4, expressed as production (g/d), yield (g/kg DMI), or intensity (g/kg milk), using recognized *in vivo* techniques (e.g., respiration chamber, SF6 tracer, or portable accumulation chamber) or a validated *in vitro* batch-culture system. Studies were excluded if they lacked direct CH^4 measurements, did not investigate nutritional strategies, or where buffalo-specific data could not be isolated in multi-species comparisons.

The article selection process followed a rigorous screening workflow to evaluate suitability and exclude irrelevant articles, applying research queries to the titles, keywords, and abstracts; subsequently, a full-text assessment was conducted to apply the inclusion and exclusion criteria. The authors emphasize that the entire database consultation and article screening process has been performed without any software or AI tools. During the gathering of information, articles providing additional data or scientific evidence were scrutinized and, if deemed relevant, included. Lastly, to not compromise the overall manuscript's readability and clarity, unless otherwise specified, results reported in the text should be interpreted as indicating statistical significance between values (p<0.05 or p<0.01). Figure **1** illustrates the literature screening and selection process. This structured approach ensured the selection of high-quality evidence to support the narrative review on nutritional interventions for enteric methane mitigation in buffaloes.

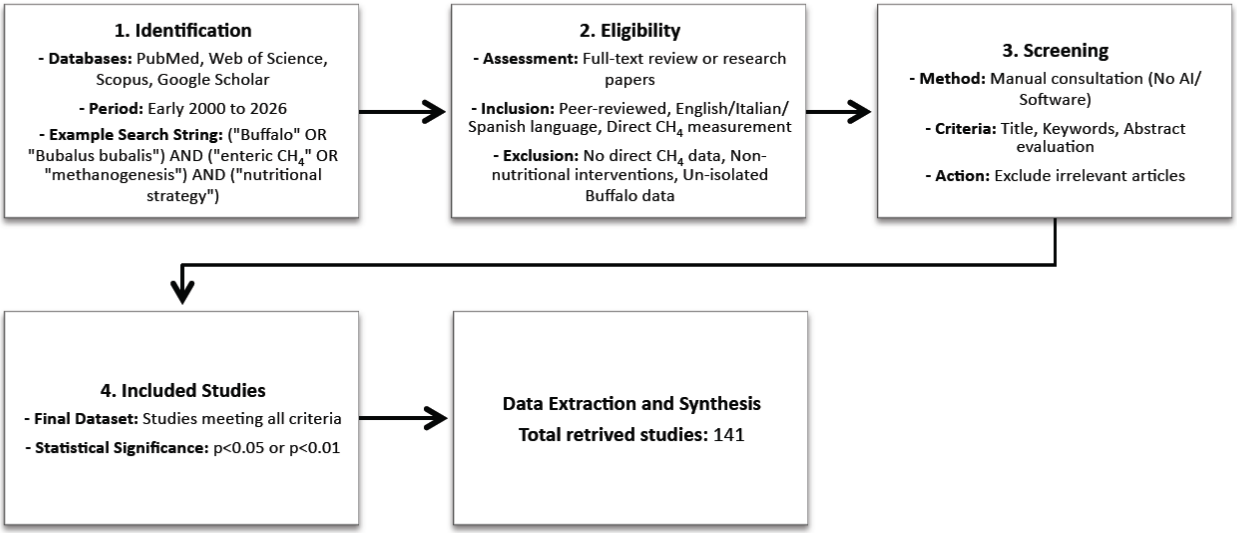

Figure 1: Flowchart of the literature identification, screening and selection criteria.

3. *BUBALUS BUBALIS* DIGESTIVE ANATOMY, RUMEN PHYSIOLOGY, AND MICROBIOTA IN COMPARISON TO *BOS TAURUS/BOS INDICUS*

As ruminant species, buffalo graze the majority of the day on human-inedible fibrous roughage, converting fiber's fermentation end-products into animal protein [27, 40]. Within the rumen, a diverse consortium of microbial populations comprising bacteria, fungi, protozoa, bacteriophages, and archaea facilitates the host organism's digestive functions related to the breakdown of complex carbohydrates and the production of VFAs and MP [29, 38]. Nevertheless, while Prevotella, Fibrobacter, and Ruminococcus spp. stand among the main bacterial communities involved in fiber degradation, inter- and intraspecific variability, physiological state, and to some degree, geographical location, may influence the relative composition of the rumen microbiota [27, 38, 41, 42]. However, the feeding regimen has been identified as the dominant factor in rumen microbiota composition [31, 38]. In addition, as buffalo and bovines belong to distinct subfamilies, they exhibit several differences, as summarized in Table **1** [43], which paved the way for specific investigations to deepen understanding of their species-specific nutritional requirements and ruminal ecosystems [3, 10, 44].

Relative to the digestive ability, their greater grass consumption hints at a ruminal microbiota more prone to roughages compared to cattle [45]. For this reason, buffaloes are considered to convert low-quality forages more efficiently than cattle [2, 46]. For instance, their digestive physiology exhibits superior nitrogen (N) recycling and lower excretion of purine derivatives, facilitating buffalo consumption of N-poor feeds [44, 47]. In addition, when fed on the same diet, the net energy intake in buffaloes has been found to be higher than that of crossbreed cattle [48]. However, when provided with grass hay (*Brachiaria ruziziensis*), other authors observed comparable energy intake, loss, and retention in buffaloes and Brahman cattle [49]. In light of the buffalo species' tropical origin, a typical diet consisting of what is available on farm, usually low-quality and fibrous feedstuffs such as crop residue, might have induced ameliorative adaptations of fiber

Table 1: Physiological and Digestive Differences between Buffalo and Cattle Influencing Digestive Physiology and Enteric Methane Production

Item	Buffalo (*Bubalus bubalis*)	Bovine (*Bos taurus/Bos indicus*)	Implications for CH_4 production	References
Digestive tract	Larger rumen-reticulum volume relative to body size and longer digesta retention time within the rumen	Slightly smaller volume and shorter residency time	Larger rumen and longer retention support greater microbial fermentation capacity and fibre degradation, but might increase methanogenesis substrates availability	[43, 51, 53, 54]
Feed utilization efficiency	More extensive mastication, specialised rumen papillae, better N utilization might contribute to better utilisation of low-quality forages/crop residues	Minor feed mastication and lower conversion rate, generally more dependent on higher-quality diets	Greater fibre fermentation may increase substrate for methanogenesis but greater absorption ability might streamline VFAs removal. Feed intake and diet composition strongly affects CH_4	[2, 42-44, 46, 52, 55, 85]
Rumen microbial population	Higher incidence of lytic bacteria and non H_2 producing bacteria (*Fibrobacter succinogenes*)	More variable composition	Microbial differences affect fermentation pathways and CH_4 yield. Buffalo shows a more intense propionate pathway which is negatively correlated with CH4 production	[42, 45, 55, 58, 59, 65, 72]
Protozoal/ Fungal population	Often higher or more active	Generally lower	Protozoa produce H_2 and indirectly promote methanogenesis due symbiotic relationship with methanogens	[45, 55, 59, 97]
Methanogenic archaea	Generally lower and less diverse	Higher abundance and diversity of CH_4 producing bacteria	Buffaloes' lower archaeal population might facilitate the design of targeted nutritional interventions	[19, 28, 29]
CH_4 production	Generally lower	Higher	Both *in vitro* and *in vivo*	[19, 64]
Response to dietary additives	Less studied; species-specific responses possible	Well-documented in various bovines breed	Caution applying cattle strategies directly to buffalo	Present study

fractions' digestion [40, 50]. For instance, buffaloes exhibit more extensive feed mastication, which may facilitate better ruminal degradation of structural carbohydrates [43, 51], and possess highly specialized rumen papillae that contribute to superior absorptive function of fermentation end-products [43, 52]. Moreover, regarding their digestive system, their larger gastrointestinal capacity and rumen-reticulum complex volume, coupled with the longer feed residency time [53, 54], might contribute to their enhanced digestion. Perhaps, compared to cattle, to compensate for the low nutritive value of tropical forages, an increased rumen–reticulum capacity enabled buffaloes to store greater quantities of feed [43]. Conversely, although the mean food passage rate of buffaloes has been observed to be shorter, the prolonged retention time of ingesta within the rumen [53] may constitute an additional adaptation that favors the fermentation of plant fiber by their ruminal microbiota, thereby underpinning ascertained microbial differences between buffaloes and cattle species [45, 55].

Indeed, buffaloes' greater ability to thrive on fiber-rich diets compared to cattle can be attributed to differences in the relative composition of the ruminal microbial community (Table **1**), which influences nutrient degradation [28, 45, 54, 55]. Notably, corroborating the early divergent establishment of ruminal microbiota, buffalo calf rumen shows markedly higher bacterial richness than that of Holstein calves under the same feeding regimen, and at the phylum level, *Firmicutes* were significantly more abundant [45]. Whereas at the species level, *Fibrobacter succinogenes* has been found to be naturally more abundant in buffaloes' rumen [55]. On the other hand, buffalo rumen has a higher abundance of *Bacteroidota* species belonging to the genus *Prevotella*, which facilitates the degradation of noncellulosic plant fibers [28, 56, 57]. The greater incidence of lytic bacteria, the higher presence of anaerobic fungi and protozoa in the buffalo rumen microbiota, along with differences in community composition compared to cattle, might contribute to the higher degradation of fiber fractions [19, 45, 51, 55, 58, 59]. Accordingly, the distinct rumen microbiota composition and activity of buffaloes also reflect on nutrients' conversion in fermentation products and relative VFAs' production, as confirmed by Iqbal *et al.* [55] observing numerically higher acetate and propionate production, and statistically lower acetate:propionate ratio (A:P) in buffalo compared to jersey cow calves. Overall, these differences translate into buffalo's digestive potential, showing greater capacity to utilize low-quality roughage and to more efficiently convert fibrous feed and crop residues into animal protein [40, 43, 45, 55, 60].

Regarding methanogen populations, some authors found that buffalo harbors a lower proportion of gas-producing bacteria (Table **1**) [19, 28, 55]. Indeed, smaller Archaea populations associated with methanogenesis are reported in buffalo compared to cattle, with <5% and around 10%, respectively [28]. Moreover, when fed a similar 70:30 Napier (Pennisetum purpureum) green grass and concentrate diet, buffaloes' archaeal community comprises 20 species of methanogens spread across 7 phylogenetic orders, showing a greater degree of heterogeneity than cattle, which harbors 25 species belonging to 6 families [19]. Notably, at the genus level, *Methanobrevibacter* constitutes the most representative methanogens identified in both species [55]; whereas, the second most abundant genus was related to *Methanomassillicoccales* in buffalo and to *Methanomicrobiales* in cattle [19]. However, contrasting dominant methanogen lineages are found within the buffalo species. For instance, ruminal communities of Indian Murrah [61] and Surti [32] water buffaloes fed forage and concentrate are largely dominated by *Methanomicrobium;* whereas, regardless of diet, buffaloes from Brazil and India showed *Methanobrevibacter*-related phylotypes as predominant [62, 63]. This suggests a decreased capacity for hydrogen (H) removal via CH_4 production, thereby conserving metabolic energy and corroborating the higher net energy intake of buffalo [48].

Accordingly, although contrasting results are reported in the literature [48], buffaloes' anatomical and microbiota characteristics are generally associated with lower CH_4 emissions as a fermentation by-product [19, 64]. In this regard, from an *in vitro* study, Mediterranean Buffalo rumen fluid generates significantly less CH_4 than bovine, either in relation to the degraded organic matter (OM) or when reported as % of total gas (respectively 75.1 *vs* 97.1 ml/g and 18.8% *vs* 20.3 %) [64]. Moreover, the energy loss as CH_4 on gross energy intake is reported to be lower in buffaloes than cattle (respectively 3.7 *vs*. 4.4%) [49]. In addition, higher *F. succinogenes* (Iqbal *et al*., 2018), a non-H_2-producing bacterium during cellulolytic fermentation, provides succinate and propionate instead, as alternative non-methanogenic end-products [65]. Malik *et al.* [19] found that male buffalo calves generated lower enteric emissions than cattle on the same diet (93.1 *vs* 141 g/day, respectively), although the latter consumed more feed (6.86 *vs* 10.5 kg/day), which might explain the higher emissions. From an environmental perspective, reducing enteric CH_4 is a recognized route to lowering global GHG emissions; however, the success of mitigation measures and any gains in production or nutritional efficiency hinge on detailed knowledge of methanogen species' abundance and distribution across ruminant populations [19, 32].

As a consequence, these anatomical [43, 52 - 54] and rumen microbiota [19, 28, 45, 55] divergences hint that mitigation strategies effective in bovines may not translate equally in *Bubalus bubalis*. In particular, the longer feed residency time and enhanced fiber-degrading capacity in buffaloes [2, 46, 53, 54] may alter the kinetics of feed additive action, potentially requiring different inclusion levels or adaptation periods compared to those in cattle, which calls for future *in vivo* research. Furthermore, the distinct methanogen community structure, characterized by lower Archaeal abundance and a different phylogenetic distribution (e.g., *Methanomassillicoccales* vs. *Methanobrevibacter*) [19, 28, 32, 55, 61], suggests that inhibitors targeting specific methanogenic pathways may yield variable reductions. Therefore, understanding these buffaloes' specific traits is fundamental to evaluating the efficacy of nutritional interventions.

4. NUTRITIONAL MITIGATION OF ENTERIC CH_4 PRODUCTION FROM BUFFALOES

Modern buffalo nutrition is based on two pillars, balanced nutrient supply and relatively low-cost feed [3]. Ensuring that buffaloes receive a balanced nutrient supply through the diet, including energy, sustains microbial protein synthesis and translates into enhanced performance, whereas inadequate feeding impairs buffalo metabolism [66]. However, despite fiber fermentation being a pivotal step in ruminants' digestive physiology, it entails CH_4 production that is proportional to feed intake, diet characteristics, and digestibility, as well as intra-individual differences [42, 67 - 69]. In fact, a positive relationship exists between structural carbohydrate breakdown and CH_4 production, as subsequent plant cell wall degradation, some fibrolytic bacteria (e.g., *Ruminococcus* and *Eubacterium*) generate H2, which methanogenic archaea use as a substrate, facilitating its removal, with CH_4 serving as a sink [19, 42, 69]. Fiber degradation is a multi-stage process [70], and considering its influence on CH_4 production [42, 69], preventing one step of structural carbohydrate fermentation may modulate VFA production and reduce the substrates for derived CH4. Among VFA, acetate production reportedly increases H availability for methanogenesis [71], whereas shifting VFA production towards propionate and the consequent decrease in A:P are usually accompanied by reduced H availability and utilization for CH_4 production [72]. Furthermore, ruminal methanogenesis, an energy-demanding process, generally results in a loss of 2-12% of total dietary energy intake [73]; thus, its reduction can enhance ruminant production efficiency. Under this paradigm, considering feed costs is a major determinant of buffalo farm profitability, and some strategies aim to reduce enteric CH_4 emission intensity by increasing productivity through better feed formulation or enhanced genetics [35, 68, 74]. To illustrate, certain investigations have indicated that specific feedstuffs, such as chicory, exhibit lower methanogenic potential than others, such as oat [68, 75].

In light of the pivotal contribution of ruminal microbiota to buffalo's digestive process, nutritional strategies may exert both direct and indirect actions on sustainability and curb methanogenesis, without prejudicing buffalo's metabolism [8, 76-78]. For instance, increasing the proportion of readily fermentable carbohydrates or lipids in the diet rather than specific feed additive supplementation can enhance productivity, thereby reducing CH_4 output per unit of product and indirectly fostering sustainability [8, 76]. In turn, by better matching protein supply to animal requirements or by including specific feed additives, nutritional practices also deliver indirect benefits for sustainability, reducing N and fermentable organic matter excretion [77, 78]. However, the distinct anatomical and physiological characteristics of the buffalo species [19, 43, 45, 55] require careful evaluation to tailor nutritional interventions to the species traits.

On the other hand, genetic selection for feed efficiency may complement nutritional measures. However, it must avoid the confounding effect of traditional metrics that may inadvertently select for larger, more mature body size and greater intake, undermining system-level achievement [35, 79]. Conversely, residual feed intake (RFI), the difference between predicted and actual DMI for maintenance and growth, may help avoid such bias, providing a selection criterion for lower voluntary intake independent of growth rate, which does not compromise performance while improving sustainability [35, 80]. In fact, low RFI is associated with efficient N usage and reduced CH_4 production in cattle and dairy cows [81]. To bridge the information gap and gather data on this relationship in buffaloes, Sharma *et al.* [35] designed a feeding trial with Murrah heifers offered ad libitum TMR and observed relevant differences between animals classified based on RFI. Authors found that low-RFI buffaloes consumed 11.6% less dry matter daily yet sustained comparable growth rates compared with higher-RFI counterparts, supporting the notion that low RFI implies less metabolizable energy for maintenance, also for buffaloes [35]. More importantly, the low RFI cohort incurred 26.47, 30.65, and 30.83% smaller CH_4 production (g/day), CH_4 intensity (g/kg DMI), and energy loss as CH_4 production (% metabolizable energy). This led the authors to suggest that the combination of targeted nutrition with selection for low RFI could offer a pragmatic pathway to lower enteric emissions without compromising performance, especially within large buffalo populations where mitigation at scale is required [35]. Lastly, the low RFI was also associated with reduced N excretion, which, in turn, indirectly supports sustainability.

Tables **2** and **3** provide an overview of the broad spectrum of nutritional strategies intended to reduce buffaloes' enteric CH4 emissions, which are discussed within the following subsections.

4.1. Methanogenic Potential of Feed Ingredients and Diet Composition

In the literature, the simplest reported method to control CH4 emissions from ruminants involves selecting the forage to include in the ration. In fact, aside from the different nutrient content, each forage generally presents a unique methanogenic potential [78, 82, 83], with forage quality (e.g., maturity and digestibility) and type (grass or legume) tied to variable CH4 emissions [68, 83]. For instance, phenological stage affects forage quality first, and mature plants tend to be more fibrous and less digestible [78]. In contrast, total digestible carbohydrate intake has also been reported to be inversely proportional to CH4 production in buffaloes [68]. Indeed, higher forage digestibility has been found to reduce CH4 emissions; however, to evaluate the extent of the reduction potential, it must be related to the animal species and the appropriate metric [84]. This is because, in both buffaloes and bovines, daily DM and OM intake are positively related to emissions, owing to greater digestible nutrient intake translating into increased CH4 production (g/day) [68, 78, 84]. Conversely, when CH4 yield was calculated based on dry matter intake (DMI) and productivity, it showed inverted relationships. In fact, CH4 production (g/kg DMI) and intensity (g/kg milk) are found to be inversely proportional to forage digestibility in dairy cows, but no difference in CH4 yield (g/kg DMI) was observed for beef cattle [78, 84].

In addition, assessing the methanogenic potential of routine feed ingredients might be an essential step toward incorporating gas-production information and formulating more sustainable diets for buffaloes that maintain adequate nutrient intake while inherently having a lower CH_4-producing capacity [68, 85]. In fact, although not statistically significant, based on digestible nutrient intake, tropical forages like chicory and berseem are found to be less methanogenic than oat in Murrah buffalo calves [68]. On the other hand, Prusty

Table 2: Comparison of the Main Nutritional Approaches and Mitigation Extent

Strategy	Reported CH_4 Reduction	Key Considerations	References
Feed efficiency selection (low RFI)	~20-30%	Long-term genetic approach	[35]
Optimized nutrient content and forage-to-concentrate ratio	~10-20%%	Must balance with rumen health	[66, 88, 89]
Higher energy density diets	~5–25%	Requires access to quality concentrates and careful diet formulation	[22, 91]
Dietary lipid supplementation	~10–40%	Less evaluated *in vivo*. Watch for reduced DMI at high inclusion levels	[8, 92-95, 98]
Phytogenic feed additives (tannins, saponins, EOs)	~5–70%	Cost-effective, improves immunity and performance, but requires careful selection of PSM to include	[63, 77, 104-106, 126]
Probiotics	~8-9%	Further *in vivo* investigations needed to identify beneficial buffalo-specific strains	[36, 74, 131, 136]
Composite feed additives	~7-75%	The combination of different approaches might augment mitigation gains, but also compromise them	[138-141]

Table 3: Effect of the Different Nutritional Approaches to Mitigate Enteric Methane Emissions in Buffaloes

Nutritional Strategy	Specific Intervention	Inclusion Rate	Experimental Context	CH_4 Reduction	Major Outcomes	Reference
Management	Selection for Low Residual Feed Intake (RFI)	Genetic Selection	Murrah heifers (*in vivo*)	26.47–30.83% smaller CH_4 production	Sustained growth rates with lower dry matter intake	[35]
Diet Composition	Sorghum silage (vs. Maize silage)	Substitution in diet	Mediterranean buffaloes (*in vivo*)	39.6% reduction in CH_4 yield	No adverse effects on weight gain or microbial population	[87]
Diet Composition	Balanced Ration (Nutrient alignment)	Adjusted to requirements	Indian lactating buffaloes (Field)	13.3–23% reduction in CH_4 yield	Increased DMI, milk production, and fat content	[88, 89]
Dietary Lipids	Palm kernel (by-product)	0.25, 0.50, 1% body weight	Growing buffalo (*in vivo*)	21–60% reduction (kg CH_4/year)	Enhanced DMI and feed efficiency; reduced carbon footprint	[8]
Dietary Lipids	Sunflower oil	0.4 and 0.8 ml/30ml rumen fluid	Buffalo rumen fluid (*in vitro*)	~37% lower CH_4 concentration	Shifted VFA profile; reduced A:P	[94]
Phytogenic feed additive	Eucalyptus and Poplar leaves	15 g/kg diet	Lactating buffaloes (*in vivo*)	37% reduction in CH_4 concentration (exhaled air)	Enhanced nutrient digestibility; no change in milk composition	[77]
Phytogenic feed additive	Phytogenic blends (EOs, Saponins, Tannins)	0.05–2% DMI	Growing buffalo calves (*in vivo*)	5.1–17.8% reduction (L CH_4/kg DMI)	Improved feed conversion; enhanced crude protein digestibility	[105]
Composite Additives	Rumen modifier blend (7 plants+Na_2SO_4)	2%+0.06% and 4%+0.06%	Buffalo (*in vivo*)	9.6–14.8% lower CH_4 production/kg DMI	Enhanced immunity markers; no impact on nutrient digestibility	[138]
Composite Additives	Commercial additive (Yeast, Capsaicin, etc.)	100 g/animal/day	Lactating buffaloes (Under heat stress)	7% reduction in daily CH_4 emission	7.4% higher daily milk yield; reduced heat stress load	[139]
Composite Additives	Lipidic+PSM+Inhibitors+Electron acceptors	106 g/day	Buffalo calves (*in vivo*)	Up to 75% reduction in CH_4 emissions	Improved body weight gains and feed efficiency; reduced N excretion	[141]

et al. [85] evaluated the *in vivo* effect on CH_4 emissions in Murrah buffalo calves fed different isoenergetic and isonitrogenous concentrate mixtures. These were formulated with the least versus the most methanogenic feed ingredients based on prior *in vitro* gas production results. Buffalo calves fed low-CH_4 diets emitted significantly less CH_4 per kg DMI (20.9 g/kg DMI and 20.7 g/kg DMI) compared to the group fed high- CH_4 ration (25.1 g/kg DMI +20.7%). Although not previously reported as significant in forage-fed calved [68], the administration of low methanogenic rations is also found to lower energy loss for methanogenesis since the energy loss as a percentage of metabolizable energy was also significantly lower in both low-CH_4 fed groups (respectively 10.9 and 10.6 *vs.* 12.9%) [85]. Regarding the forage source, maize silage has been found to lower CH_4 production (g/kg DMI) in dairy cows, possibly due to differences in feed kinetics or a ruminal fermentation shift toward propionate formation [86]. However, the higher fermentable OM content of feces, which rather contributes to indirect CH_4 release [78, 84], along with its more water-intensive production [13], makes its use unsustainable. On the other hand, substituting maize silage for sorghum silage in the

Mediterranean buffaloes' diet scored a significant 39.6% reduction in CH_4 yield (respectively 103.00 kg and 63.48 kg CH_4/head/year) with no observed repercussions on weight gain or microbial population [87].

Nevertheless, when designing mitigation strategies intended for buffaloes, apart from being tailored to buffaloes' digestive physiology, they should also consider the rearing context [2, 3], rather than assuming cattle-based forage recommendations as valid. Indeed, in many tropical regions where buffalo rearing is prevalent, the availability of specific low-methanogenic forages, such as chicory or sorghum silage [68, 87], is often limited or seasonally dependent. Consequently, the economic viability of these ingredients must be weighed against the potential reduction in enteric emissions. Therefore, mitigation strategies relying on specific feedstuffs must be evaluated not only for their biological efficacy but also for their on-field feasibility and cost-effectiveness within diverse rural or large-scale production systems [68, 85].

4.2. Nutritive and Energetic Content of Diet

An evaluation of feeding regimens across Indian buffalo farms revealed that diet composition and nutrient supply vary depending on the region [88]. Discrepancies between dietary nutrient intake and actual requirements result in inadequate energy metabolism and MP synthesis, compromising productivity and increasing environmental burdens [66, 88]. Therefore, aligning ration's nutritional content with requirements has been proposed not only as a strategy to promote nutrient availability and production, but also for reducing CH_4 emissions in lactating buffaloes [66, 88, 89]. Indeed, under field conditions, adjusting the diets of Indian lactating buffaloes to ensure a fair nutrient supply showed a ~13.3, 23, and 18% reduction in daily CH_4 yield, DMI-related emissions, and CH_4 production as % energy intake, respectively, alongside increased DMI, milk production, and fat content [66]. Likewise, Sherasia *et al.* [88] investigated the benefits of balanced ration implementation across the Indian region. Findings revealed overall improvements in milk production and efficiency, along with a mitigating effect on various CH_4-emission-related indicators (g CH_4/day, g CH_4/kg milk, and % dietary energy loss as CH_4). For instance, the all-regions average daily milk yield increased by 7.5%, and production efficiency (kg fat-corrected milk/kg DMI) increased by 9.6%, whereas CH_4 production per kilo of milk decreased by 12.6% and the share of total energy employed in CH_4 production decreased from 5.7 to 4.9% [88]. More recently, Sherasia *et al.* [89] evaluated the effectiveness of ration balancing in crossbred dairy cows and Murrah buffaloes. In addition to results similar to those of previous studies, such as increased nutrient supply and milk production, and decreased CH_4 emissions (g/day and g/kg milk), authors also reported a significant enhancement of immunity, along with -14.3 and 18.4% lower milk production costs, respectively for dairy cows and buffaloes [89].

In the same way, the metabolizable energy content of the ration has also been found to influence CH4 emissions in growing buffalo heifers [22]. Indeed, low dietary metabolizable energy intake was associated with greater growth inhibition, increased CH4 production (g/day, g/kg DMI), and greater energy loss (% energy intake) compared with standard or higher metabolizable energy intake. In addition, the same study revealed that, regardless of season, buffalo heifers fed a high-energy diet emitted less CH4 [22]. The authors proposed that the higher CH_4 production could be explained by the increased inclusion of wheat straw in the low-energy group, and its low fiber digestibility [22]. Indeed, low carbohydrate digestibility increases CH_4 production [68, 78]. Likewise, providing surplus dietary energy content has been assessed as a viable approach to significantly mitigate CH_4 emissions (g/day and g/kg DMI) in tropical Brahman cattle [90]. Moreover, although not elucidated by Talukdar *et al*. (2017), the elevated dietary energy content might also have induced a shift in the rumen fermentative pattern, thereby achieving CH_4 mitigation. In fact, raising dietary energy content by increasing concentrate and decreasing fiber inclusion promotes ruminal propionate production rather than acetic acid, which is negatively correlated with CH_4 production [22, 72].

Thereby, balanced feeding and reduced dietary energy excess might sustainably enhance buffalo production by lessening CH4 emissions [68, 88, 89]. Balanced buffaloes' feeding optimized nutrient supply and influenced rumen fermentation in favoring the synthesis of MP over VFA, as supported by the increased microbial N flow [68, 88,89]. In fact, MP and CH4 production have been found to share an inverse relationship [79]. In turn, the higher starch content of an energy-dense diet might contribute to a decrease in CH_4 production by shifting rumen fermentation towards more propionate [22, 72]. Nevertheless, while the theoretical benefits of ration balancing are well documented [68, 88, 89], its practical application within commercial systems warrants critical scrutiny. In many tropical regions where buffalo rearing is prevalent [2, 4], sourcing concentrate feed or mixtures is often difficult or expensive. Furthermore, the majority of the global buffalo population is reared by smallholder farmers, and implementing balanced rations often requires infrastructure for feed mixing and storage that may not be accessible. In rural contexts, buffalo's diet costs unfortunately represent a higher priority than CH_4 mitigation.

4.3. Dietary Forage to Concentrate Ratio

Lastly, provided concentrate feeds' ruminal fermentation changes the relative VFA balance, yielding more propionate [72], diet composition in terms of the forage-to-concentrate ratio (F:C) has also been found to affect enteric gas production. As a matter of fact, although methanogen populations are little influenced by the amount of roughage consumed, high fibrous-feedstuffs intake results in augmented CH_4 emission [32]. However, meticulous diet formulation is needed to prevent the negative health outcomes of ruminal acidosis caused by excessive dietary carbohydrates in dairy ruminants [14]. Investigating the methanogenic potential of different F:C ratios in buffaloes and crossbreed cattle, Sinha *et al.* [48] observed that a high concentrate diet results in a significantly lower energy share being lost to CH_4 production than a high roughage diet, respectively, 5.48% and 7.91% of the gross energy intake. Similarly, in growing buffalo calves, the administration of varying F:C diets (F:C at 80:20, 60:40, 40:60) yielded different results in terms of animal performance and both direct and indirect GHG emissions (enteric CH_4 and fecal N_2O) [91]. Notably, higher concentrate intake in the 40:60 F:C group resulted in greater daily weight gain than in the high forage diet, despite the trial recording no variation in DMI or feed efficiency ($p>0.05$). This same group also exhibited a -21.5, -30.37, and -40% reduction, respectively, in daily ruminal CH_4 yield (g/day), CH_4 production (g/kg DMI), and CH_4-energy loss (% metabolizable energy intake) compared to the high forage-fed group. Nevertheless, the intermediate group did not achieve statistical significance [91]. However, investigating GHG fluxes from calves' storage revealed that high-concentrate diets led to a significant +61.9% increase in N_2O (mg/kg DM) emissions compared to low-to-moderate concentrate inclusion levels [91], which might offset the mitigation gains from decreased ruminal CH_4 production.

While increasing the F:C can shift VFAs production towards propionate and reduce CH_4 yield [72], this strategy must be applied cautiously in buffaloes. Their physiological adaptation to high-fiber diets [2, 45] and specific rumen environment [19, 28, 45, 55] may render them more susceptible to ruminal acidosis if concentrate inclusion is increased too rapidly, potentially negating mitigation gains through compromised animal health and productivity [14].

4.4. Lipids and Oilseeds

Fat supplementation is a straightforward method to increase the ration's energy content while avoiding excessive intake of fermentable carbohydrates (Patra, 2013; Dey *et al*., 2018, 2020). Furthermore, previous investigations have recorded decreased CH_4 yield when lipid sources were increased in the ration, both *in vitro* and *in vivo* [8, 92 - 95]. This might be related to the biohydrogenation process, which involves the progressive saturation of dietary unsaturated fatty acids by ruminal microflora, serving as an alternative H sink instead of CH_4, or to the recorded lower A:P in bovines [96]. In addition, protozoa are reputed to live in an endosymbiotic relationship with methanogens [97], and Wanapat *et al*. [98] confirmed that *in vivo* vegetable oils supplementation induced a decrease in buffalo's protozoal count, which might contribute to lower CH_4 emissions. However, the mixed results of fat supplementation on gas production reported in the literature might be explained by different lipid sources, compositions (saturation and chain length), and inclusion rates [93, 98]. For instance, despite both sesame and mustard oils *in vitro* inclusion producing a curb in CH_4 production (ml/g DM), mustard oil's reduction of CH_4 concentration outperformed that of sesame's [83]. In addition, Dey *et al*. [94] reported that *in vitro* sunflower oil inclusion at two dosages (0.4 and 0.8 ml/30ml buffalo rumen fluid) resulted in an average 37% lower CH_4 concentration and that the higher inclusion rate also significantly reduced CH_4 production (ml/g DM). Although more *in vivo* buffalo-specific trials are required, this observation suggests that lipid supplementation *in vitro* impairs MP production and shifts the fermentation pattern towards less acetate production, resulting in lower A:P [92-94]. In both studies, the authors hypothesized that *in vitro* fat supplementation might have suppressed methanogenesis by altering ruminal microbiota or impairing feed digestibility [93, 94]. Notably, protected fat supplementation in buffalo rumen fluid (up to 30 g/kg DM) did not adversely affect *in vitro* DM or nutrient digestibility, while it linearly increased total VFAs concentration [99]. Still, despite the fact that methanogenesis-related implications were not evaluated, protected fats as an energy-dense supplement might reduce *in vivo* CH_4 emissions by improving productivity without altering rumen fermentation [99].

Recently, Li *et al*. [95] investigated the *in vitro* effect of different dosages of unsaturated fatty acids (1, 3, 5, and 7 % DM) on a 70:30 elephant grass and a standard concentrate diet. Linoleic and linolenic acids were tested individually on the ruminal population and on fermentation kinetics, including relative and total VFAs production, A:P, and the impact of their supplementation on methanogenesis. In accordance with previous reports suggesting differential results might be due to lipid source characteristics [93, 98], the authors found the two fatty acids to induce similar significant results at different concentrations, hinting at different degrees of influence on the parameters under investigation [95]. Namely, similar results were found regarding CH4 production, which was significantly reduced at concentrations above 3% for both fatty acids, despite linolenic acid displaying major CH4 mitigation (-24.52 vs -60.61% ml CH_4/g DM at 3% inclusion level). This difference underscores the diverse influence of fatty acid saturation on biohydrogenation kinetics [95, 98]. Furthermore, linolenic acid influenced single- and total-VFA production, reducing acetic acid production and A:P owing to the concomitant increase in propionic acid, at concentrations above 1%, while linoleic acid induced similar results at 3% [95]. However, the diverse effects of the two fatty acids might also be due to their differential effects on ruminal microbial populations. In fact, linolenic acid significantly reduced methanogen populations at 3% (-47%), whereas linoleic acid had no effect. Moreover, negative effects on protozoa were observed at concentrations greater than 1% and 5%, respectively, for linolenic and linoleic acids [95].

Nevertheless, literature regarding lipid *in vivo* administration in buffalo's diet on methane production implication is somewhat lacking, and further studies are warranted to delve into the *in vivo* mitigation potential of lipid supplementation in buffaloes, owing to a significant reduction in both DM and fiber digestibility being witnessed as vegetable oil was included *in vitro* [93, 94]. Notably, increasing the inclusion rate of palm kernel (0.25, 0.50, and 1% based on body weight) in the buffalo diet was investigated for its effects on productive and CH4 production-related parameters [8]. The supplemented group achieved enhanced DMI and feed efficiency, lowering *in vivo* enteric CH_4 emissions by 21, 18.6, and 60% kg CH4/year, respectively, which contributed to a lower overall meat carbon footprint of -34.33 CH_4/kg meat [8]. From a production perspective, reducing the meat carbon footprint signifies a valuable step for the environmental sustainability of the buffalo sector.

However, the implementation of lipid supplementation in buffalo nutrition to mitigate methanogenesis is closely tied to the lipidic source and ruminal environment [45, 93, 98]. The current scarcity of *in vivo* buffalo-specific data [8] limits its practical application and leads to frequent extrapolation of inclusion rates from cattle studies, which poses an inherent risk given the species' distinguishing features. Namely, buffaloes possess distinct digestive physiology, including prolonged digesta retention time, and a unique ruminal consortium, characterized by greater microbial activity, lower methanogen abundance, and different community composition [19, 28, 45, 53-55]. Such a different physiological framework suggests variable *in vivo* results, as the extended retention time and different rumen microbiota may induce a higher degree of fatty acid biohydrogenation and consequent H diversion away from methanogenesis, which may differ considerably from cattle. Additionally, although lipid supplementation is associated with reduced CH_4 yield, high inclusion rates of vegetable oils have at times been reported to impair DM and fiber digestibility [93, 94]. Therefore, the balance between mitigation efficacy and nutrient availability calls for further *in vivo* studies and requires careful species-specific optimization to avoid compromising health or productive performance. Indeed, considering buffaloes' greater tendency for high-fiber diets [45], a rapid increase of the ration's lipid content may more severely increase the risk of ruminal acidosis compared to cattle [14].

4.5. Phytogenic Feed Additives

Besides diet composition and lipid supplementation, another class of nutritional strategies involving plant secondary metabolites (PSMs) can exert a positive influence on the microbial community through antimicrobial activity [31]. PSMs, including phenols, tannins, saponins, and essential oils (EOs), are ubiquitous in the plant kingdom, including feed resources and agro-industrial by-products, and, at appropriate doses, are known to exert antimicrobial and health-promoting effects [31, 100]. Indeed, mounting scientific evidence links the inclusion of specific quantities of PSMs in ruminants' diet (either through PSMs-containing feedstuffs or purified feed additive), to a vast array of influences on rumen environment related to microbial community modulation, carbohydrate and protein degradation, and unsaturated fatty acids biohydrogenation [31, 77, 101, 102]. Yet, livestock intensification has simplified ruminant diets through the use of cultured pastures and TMR, reducing dietary PSMs that support animal health and enhancing meat and milk quality [31, 77, 103]. In particular, administration of eucalyptus and poplar leaves in lactating buffaloes has been associated with improved N metabolism and enhanced cell-mediated and humoral immune responses [77].

Notably, the methanogenesis mitigation potential inherent in the PSM content of several plant species also prompted investigations aimed at reducing environmental degradation, although a substantial portion of these inquiries focused on cattle [24, 31, 85, 104, 105]. Nevertheless, within literature, numerous PSMs found in seeds, leaves, flowers, buds, and bulbs containing EOs, polyphenols, and saponins, are pointed at as potential feed additives in buffalo nutrition due to *in vitro* methanogenesis regulation (e.g., Sapindus mukorossi and S. saponaria, Populus deltoides, Mangifera indica, Allium sativum, Salvia officinalis) [106, 107]. In accordance, the administration of a phytogenic-based feed additive has recently been *in vivo* evaluated in lactating buffaloes, achieving a significant reduction in CH_4 concentration in exhaled air [77]. These findings underscore the practical potential of phytogenic inclusion in buffalo diet. PSMs-induced modifications represent a valuable tool for methanogenesis mitigation, with the literature generally reporting reduced VFAs production, especially acetate, through direct inhibitory action on ruminal fibrolytic bacterial and protozoal populations [31, 77, 108].

Indeed, PSMs exhibit *in vitro* and *in vivo* antimicrobial activity against methanogenic Archaea (e.g., Methanobacteriales), thereby contributing to reduced methanogenesis [106, 109, 110]. Therefore, the antimicrobial activity of phytochemicals could be considered a potential tool to induce a selective modulatory effect, targeting the abundance or presence of specific microbial populations in the buffalo rumen, which warrants future *in vivo* studies to assess the extent of mitigation [31, 106]. However, Kamra *et al*. [106], in a review of the *in vitro* CH4-Reducing potential of 31 plant extracts (methanol-, ethanol-, and water-based) and their effects on buffalo rumen microbes, found heterogeneous results. Inhibition rates of the screened samples ranged from little to none to more than 80%. Still, only 20 extracts from 14 plants were found to induce a notable depression in CH_4 production, highlighting the need to prioritize investigating plant sources with high mitigation potential for commercial application. Authors proposed that not every PSM displays equal *in vitro* anti-methanogenic potential; in addition, some effective extracts were water-based, hinton-water-soluble, and mostly non-water-soluble [106, 107]. In accordance, not every extract reduced ruminal methanogenesis to the same extent, meaning only some PSMs simultaneously depress CH4 production while inhibiting ciliate protozoa growth. Regardless, plant extract-induced CH4 reduction was usually accompanied by a simultaneous decrease in feed digestibility, except for the extracts of Allium sativum, Foeniculum vulgare, and Mangifera indica, which warrant further studies to deepen understanding of *in vivo* outcomes [106].

Among PSMs, tannins, classified as condensed (CT) or hydrolyzable (HT), act as molecular modifiers of ruminal fermentation, reducing CH_4 emissions through direct inhibition of methanogenic bacteria and protozoa, as well as by functioning as electron acceptors [31, 110 - 112]. For instance, forage legumes are rich in PSMs (especially tannins), whose intake, aside from enhancing ruminants' productivity, is also reported to exert anti-methanogenic effect [31, 85]. Indeed, dairy cows fed on legume forage silage (*Lotus corniculatus*) showed a significant 24% decrease in CH_4 emission intensity (g/kg DMI) in comparison to the ryegrass silage control diet [113]. Furthermore, investigation delving into the mechanisms underlying tannin-induced CH_4 reduction involves impaired fiber fermentation, which limits substrate availability [109]. In fact, the methanogenic-decreasing potential stems from reduced fiber degradation caused by tannins, which prevent microbial adhesion to plant cell walls or alter their function or enzymatic profiles [114, 115]. Nevertheless, despite extensive inquiry, the precise pathways by which tannin administration interferes with ruminal methanogenesis and curbs emissions remain to be elucidated, with no definitive empirical corroboration [112]. In fact, while both tannin classes depress VFAs concentrations, HT and CT have been associated with differential behavior against ruminal microorganisms, and each type induces distinct compositional responses via selective regulation of fibrolytic bacterial communities [112, 116]. Nevertheless, comparing the antimicrobial activity of individual tannin classes reveals that tannin type governs microbial population dynamics and, as a downstream consequence, the final VFAs profile.

Specifically, CTs are often reported to be relatively ineffective against Gram-negative bacteria compared with Gram-positive strains; on the other hand, HTs are more prone to ruminal microbial cleavage [116], suggesting a fiber digestion-independent mechanism of action [31].

Notably, in addition to tannins, EOs and saponins represent additional groups of valuable phytochemicals investigated for their capacity to modify the rumen environment and mitigate CH_4 emissions [31, 107]. Scientific evidence on saponin-containing plants indicates that these exhibit *in vitro* activity against protozoa, facilitating the mitigation of methanogenesis [106, 107, 117]. For instance, *Yucca schidigera* extract has been reported to significantly decrease *in vitro* CH_4 yield, A:P, and protozoal counts without altering DM digestibility or VFAs production [118-120]. Whereas *S. sapindus saponaria* induced a 20% reduction in $CH_{4,}$ attributed to decreased protozoal populations, without affecting bacterial or methanogen populations or fiber digestibility [121]. Supplementing cattle feed with 60 g/day of quillaja extract, a saponin-rich plant, resulted in a 61% drop in protozoal count [122]; in exchange, *in vitro* inclusion of *Sapindus mukorossi* extract in buffalo rumen liquor displayed methanogenesis and ciliate protozoa inhibition [107]. However, other saponin-rich plants, such as *Equisetum arvense* and *Salvia officinalis*, negatively impacted methanogenesis without observable effects on protozoa [123]. Regarding the mode of action underlying saponins' antiprotozoal activity, their binding to cholesterol in protozoal cell membranes and their induced cell lysis might support this effect [124]. Given that the literature mentions a symbiotic relationship between methanogens and ciliated protozoa [97], saponins' adverse effects on H-producing ciliate protozoa and cellulolytic bacteria suggest that reduced CH_4 production may result from limited substrate availability for the methanogenesis process [106, 125].

4.6. *In Vivo* PSMs Inclusion in Buffalo Diet

To fill the gap in *in vivo* trials, many ruminant scientists investigated the effects of administering PSM-containing essences in buffalo species [63, 77, 105, 126]. For instance, different phytogenic feed additives were assessed in growing buffalo calves over an eight-month trial to deepen understanding of their differential effects. These additives included mixtures of EOs, saponins, and tannins at varying ratios and inclusion levels [63, 105]. Although not statistically significant ($p>0.05$), following administration of different phytogenic blends, a reduction in methanogenesis was observed [105]. The three feed additives included ajwain and lemon grass oils (1:1 at 0.05% DMI), garlic and soapnut (2:1 at 2% DMI), and garlic, soapnut, harad, and ajwain (2:1:1:1 at 1% DMI). Authors reported that L CH4/kg DMI and L/kg digestible DMI in the experimental groups were 13.3 and 17.8, 10.9% and 13.5, and 5.1% and 9.8% lower than the control for groups 1, 2, and 3 [105]. Furthermore, in a separate trial, the same research group clarified phytogenic blends 2 and 3 conditioned protozoal populations' significant decrease, hinting at antiprotozoal activity of PSMs contained in such plants [63]. Similar findings about PSMs' ability to curb methanogenesis in buffaloes were found by Inamdar *et al*. [126], which included 100 g/kg saponin-rich deoiled mahua cake in concentrate and investigated the *in vitro* and *in vivo* fermentation in buffaloes. Saponin inclusion led to statistically significant results in *in vitro* CH4 mitigation, but authors noted only a numerically decreased *in vivo* CH4 emissions; the lack of significance in this latter trial has been attributed to hypothetically low inclusion level [126]. In addition, within the same study, adding harad to mahua cake did not confer a further *in vivo* mitigation advantage among experimental groups, unlike in the *in vitro* trial [126]. However, in beef heifers, Cardozo *et al*. [127] found that supplementation with specific EOs resulted in mixed outcomes, at times increasing or reducing feed intake and/or individual VFAs production. In addition, in beef heifers, high inclusion levels of some EOs (e.g., cinnamaldehyde) negatively affect nutrient intake and digestibility [128]. Remarkably, Samal *et al.* [105] report the mitigation effect of phytogenic feed additive inclusion came with no repercussion on buffalo's feed utilisation, instead all three PSMs-containing feed additives showed to overall numerically enhance feed conversion +8.9%, as also confirmed by the improved crude protein digestibility and the positive N balance and inferior faecal N content to control [105] and no detrimental effect on rumen environment [63].

Similarly, Dey *et al.* [77] evaluated the administration of a phytogenic-based composite feed additive (15 g/kg diet) composed of 50:50 eucalyptus and poplar leaves, both rich sources of tannins, phenolic compounds, and saponins in lactating buffaloes. CH_4 concentration (ppm) in exhaled air was numerically reduced at 60 days of phytogenic feed additive dietary inclusion in buffalo concentrate, achieving significant ($p<0.05$) 37% mitigation at 90 days of experimental feeding compared to the control group [77]. CH_4 reduction in eructed air might hint at a modulatory effect of PSMs on ruminal fermentation, as corroborated earlier by Kumar [129], who incubated *in vitro* oat hay with eucalyptus and poplar extracts, recording an inhibition of methanogenesis and an increase in propionate production [129]. Similar results were found in cattle fed increasing levels of eucalyptus leaf meal supplementation (0, 100, and 200 g/day), significantly reducing CH_4 production (mmol/L) by approximately 17% and 27%, respectively [108]. In support of this finding, the authors point at significantly altered VFAs production and microbiota composition. In fact, eucalyptus leaf meal supplementation reduced acetate in favor of propionate production, implying lower A:P, and suppressing rumen proteo- and fibrolytic bacteria, along with protozoa populations [108]. However, while these findings hint at a plausible mechanism, buffalo's specific response needs to be verified, as high inclusion of phytogenic feed additives was associated with reduced feed and nutrient intake [128]. In turn, the low inclusion rate or a synergetic effect between eucalyptus and poplar leaves' PSMs could explain the similar ($p>0.05$) feed intake between groups, observed by Dey *et al*. [77]. Moreover, the experimental diet exhibited increased digestibility of DM and organic matter. Lastly, authors observed a significant increase in the dietary content of digestible nutrients, which might support the resulting increase in productivity, as evidenced by daily milk yield, 6% fat-corrected milk, and fat-and-protein-corrected milk, with no change in milk composition [77].

PSMs offer a promising yet complex potential to modulate rumen microbiota and fermentation pathways to improve buffaloes' wellbeing and productivity, and to mitigate enteric CH_4 [77, 105, 106, 126]. However, while *in vitro* screening revealed consistent statistical reduction rates, at times exceeding 80% for specific extracts [106, 107], *in vivo* efficacy yields heterogeneous results (5.1-34%), often without statistical significance [77, 105, 126]. Either way, albeit mixed *in vivo* results may be due to PSM chemical structure, concentration, and inclusion level [31, 102], protozoal inhibition and alteration of VFAs are reputed to be the most likely pathways [31, 63, 106]. However, caution is warranted as high inclusion levels, in turn, compromise feed digestibility [108, 128]. Thus, given the narrow therapeutic window, precise PSM dosing to balance environmental benefits with productive performance warrants further in-depth *in vivo* studies, as indiscriminate application may negate gains through reduced feed efficiency. In addition, despite the CH_4 reduction outcomes, the commercial deployment of phytogenic feed additives faces significant challenges regarding the composition of extracts, the definition of inclusion levels, and economic implications. Variable concentrations of PSMs in plant sources [31, 77, 106] complicate the formulation of standardized commercial products, potentially leading to suboptimal dosing under field conditions. In addition, mitigation strategies relying on complex formulations or expensive additives may be impractical. Indeed, the cost of phytogenic feed additives may outweigh the benefits of improved feed efficiency or reduced CH_4 intensity, especially within low-margin production systems widely common in rural Asia. Future research in this field should therefore prioritize cost-benefit analyses that account for the price of PSM-containing plants and the potential trade-offs between mitigation efficacy and farm rentability to ensure that mitigation strategies are also economically sustainable and practically viable for both rural farmers and commercial systems.

4.7. Microbial Approaches

Biological feed additives, such as specific probiotic strains or associations, have also been investigated in large ruminants for their ability to positively influence rumen environment and their CH_4 mitigation potential [24, 130-134]. Probiotics are in-feed included beneficial bacteria or bacterial products (lactic acid bacteria, yeasts, fructans) that, when consumed at adequate levels, confer health- and productivity-promoting properties [130, 132]. Indeed, investigations into the efficacy of probiotics in bovine and buffalo diets revealed that their inclusion yields numerous benefits for feed efficiency and milk quality, modulates the ruminal microbiota, and is associated with lower CH_4 emissions [24, 74, 130, 132, 135]. For instance, buffalo calves supplemented with either *Bacillus subtilis* or lactic acid bacteria's strains showed improved feed digestibility, increased weight gain, and growth performances compared to animals fed a basal diet [74, 135, 136]; although no such effect was observed in growing buffalo subjects [36]. Moreover, Mousa and Marwan [136] report enhanced VFAs production and reduced protozoa presence in *Bacillus subtilis*-fed buffalo calves. Therefore, by improving nutrient digestibility and reducing protozoal populations without compromising VFAs production [136], or favoring propionate's pathway [130], probiotics' administration may represent a viable strategy to facilitate CH_4 emissions' reduction [36, 131, 133].

The implementation of a probiotic blend and live yeasts has recently been investigated in buffalo nutrition for their influence on productive and sustainability parameters of growing and lactating animals [36, 133]. Live yeast inclusion in heat-stressed lactating buffaloes' diet in the amount of 5g/day produced a numerical ($p>0.05$) reduction in CH_4 production, emission intensity in CH4, and emissions per kg of produced milk (l CH4/kg milk) [133]. In particular, the addition of live microorganisms to the diet resulted in enhanced milk productivity parameters compared with the control, including daily milk yield and 7% fat-corrected milk. Accordingly, such improvements increased the farm's economic profitability and reduced feed costs [133]. The reported insignificance of buffalo's enteric CH_4 production following yeast supplementation is in accordance with Oh *et al*. [137], which observed a similar increase in milk yield but no significant or numerical mitigative effect on dairy cows' methanogenesis.

Anyhow, most of the scientific literature exploring the implementation of live feed additives in ruminants' diets, their influence on the rumen environment, and enteric CH4 emissions mitigation, focused on the most common species, such as cattle and dairy cows [24, 130, 134, 137]. On the other hand, investigation in the field of buffalo nutrition is somehow fragmented, lacks a holistic approach, or omics sciences-derived data, such as ruminal populations or phylogenetic markers-related studies to deepen available knowledge [131, 132]. Nevertheless, inconsistent results may be due to multiple factors, including inadequate strain or dosage selection, low post-ingestive survival, persistence, and rumen colonization [36, 130]. Therefore, to tap into probiotics' potential as a mitigation strategy to reduce CH_4 emissions from buffaloes, further *in vivo* studies are required to evaluate their post-ingestive survival rates and their impact on VFAs production (e.g., favoring propionate production).

4.8. Composite Feed Additives

Several investigations evaluated the combination of two or more nutritional modifications (e.g., PSM, alternative H sinks, probiotics) to elucidate the possible magnifying effect on buffalo rumen microbial communities and CH_4 emissions from composite feed additives [138, 139]. In this regard, Lakhani *et al.* [138] investigated the inclusion of a rumen modifier blend of 7 plants, including mahua seed cake, harad, ajwain, and fennel seeds at different ratios, and sodium sulfate as an alternative electron acceptor at two inclusion levels, respectively 2% + 0.06% and 4% + 0.06%. Dietary administration of the two levels of feed additive affected methanogenesis in a dose-dependent manner, leading to 9.6 and 14.8% lower CH_4 production/kg digestible DMI and 13.4 and 11.1% reduced energy loss for CH_4 synthesis compared to control [138]. Furthermore, as DMI, nutrient digestibility, and N metabolism were similar between the experimental and control groups, phytogenic PSM sources coupled with sodium sulfate might be able to depress methanogenesis while supporting buffalo wellbeing, as evidenced by the significantly enhanced humoral and cell-mediated immunity markers recorded [138].

More recently, Petrocchi Jasinski *et al.* [139] evaluated in buffaloes two inclusion levels of a commercial composite feed additive (100 and 200 g per animal per day), originally developed to support rumen function, health, and production in dairy cows, combining brewers' yeast, capsaicin (chili's PSM), *Yucca schidigera*, calcium carbonate and garlic in lactating buffaloes under heat stress conditions. Compared to the control group, the low supplementation level resulted in a 7% reduction in daily methane emissions (g/day); moreover, it was associated with greatly enhanced nutrient digestibility coefficients, translating into a 7.4% higher daily milk yield [139]. Although direct comparisons are difficult due to the lack of investigations, similar results were found when investigating the components of individual feed additives. In fact, *Y. schidigera* extract *in vitro* inclusion was found to reduce CH_4 yield without producing a deleterious effect [120, 125]. Similarly, Orzuna-Orzuna *et al.* [140] found that capsaicin induced amelioration of fiber degradation, favoring cellulolytic bacterial populations (e.g., *Fibrobacter* and *Ruminococcus*). However, the higher inclusion of the composite feed additive worsened effects on all of the above parameters, leading the authors to conclude that 100 g/day may aid buffaloes in lessening heat stress load, which, in turn, is known to impinge on health and productivity [139, 141]. Given that the majority of the buffalo population is reared in Asia, developing probiotic-based nutritional strategies in addition to lower CH4, might also facilitate buffaloes in coping with heat stress [139]

Lastly, based on *in vitro* preliminary findings [142] to evaluate the best combination of different lipidic and PSMs sources, EOs, methane inhibitor, and alternative electron acceptors (sodium nitrate and magnesium sulfate), it has been *in vivo* tested at 106 g/day in buffalo calves on growth performances and methanogenesis implications [37]. The composite feed additive produced up to a 75% reduction in CH_4

emissions, decreased N excretion, and led to more favorable body weight gains and feed efficiency [37]. Moreover, within the same study, 239 g/day of the same supplement was investigated in adult buffaloes to examine its influence on rumen consortium composition. Although the authors found bacterial and fungal presence to be similar, phylogenetic analysis revealed clues of a possible rearrangement of ruminal microbial populations following composite feed additive administration, owing to lower methanogen populations in the treatment group, corroborating the high mitigation potential observed in the calves' trial [37].

5. Research Gaps and Future Work

Despite the extensive body of work investigating nutritional strategies to mitigate enteric CH_4 emissions from ruminants, a notable gap emerges in *in vivo* trials from buffaloes that account for their specific physiological traits [39]. It is important to acknowledge that direct, head-to-head comparison of nutritional interventions in buffaloes and bovines is lacking. No single published study has directly evaluated the CH_4 mitigation efficacy of the exact same nutritional strategy on buffaloes and bovines simultaneously within a single comparative experimental design. Most literature on CH_4 mitigation research is species-specific, often due to distinct husbandry systems, geographic locations, research aims, and intra-individual differences [8, 45, 64, 77]. What exists are reviews or comparative studies that rigorously explored the physiological and rumen microbiological divergent traits of buffaloes and bovines fed on the same diet [19, 45, 55] or reviews evaluating various mitigation strategies, including dietary lipids, PSM, and probiotics across ruminant species [24, 31, 76, 78]. Nevertheless, while direct comparative trials are absent, several studies provide baseline physiological data that inform potential differences in fermentation pathways and reflect mitigation potential. Indeed, although not focused on assessing a CH_4 mitigation strategy, research comparing rumen ecology in cattle/dairy and buffalo calves under a similar feeding regime provides insights into microbial community structure and baseline CH_4 emissions [19, 45, 55].

Retrieved scientific evidence elucidated key differences between buffalo and bovine species [19, 28, 43, 45, 55]. Therefore, it may be assumed that the extent of mitigation by the same nutritional strategy currently recommended for cattle may not be equally effective or safe for buffaloes and may yield different magnitudes of results due to species-specific rumen microbiology and fermentation kinetics [19, 28, 45, 55]. For instance, buffaloes show greater consumption and conversion rates of fibrous feed compared to bovines [2, 45, 50]. Thus, mitigating CH_4 emissions via enhanced forage digestibility might deliver additional gains when implemented in buffaloes [68, 78, 83]. Contrariwise, being buffalo more prone to roughage consumption, increasing the share of concentrate in the diet to shift VFAs production and abate CH4 emissions, might compromise its digestive physiology more quickly than bovines [14]. Similarly, the antimicrobial activity of phytochemicals, particularly their ability to inhibit protozoa and methanogenic Archaea [63, 106, 109], must be evaluated within the context of the buffalo's unique rumen microbiota [19, 28, 43, 45, 55]. In particular, buffaloes harbor a lower baseline presence of methanogens [28] and display distinct community composition [19]. These characteristics could potentially dictate different sensitivities to PSMs; thus, the dose-response relationship may vary compared to cattle. Furthermore, strategies relying on direct inhibition require precise dosing to avoid suboptimal efficacy or unnecessary alteration of buffalo metabolism.

6. CONCLUSIONS

Nutritional strategies hold clear potential to curb enteric CH_4 production from buffaloes, yet their efficacy is inextricably linked to buffalo-specific anatomical, physiological, and microbial traits, as well as to experimental design. Lipid and PSM supplementation, for instance, achieved significant CH_4 mitigation *in vitro* but not *in vivo*, and strategies currently implemented in bovines cannot be assumed to exert the same effects in buffaloes. The lack of direct head-to-head comparisons means optimal dosages are currently extrapolated from bovine data, carrying significant risks for animal health. Therefore, to optimize CH_4 reduction, species-specific physiological traits and the heterogeneity of production systems must be considered. Future research should prioritize: i) comparative trials where cattle and buffaloes are fed identical basal diets and additives under a single experimental design; ii) long-term *in vivo* trials to validate short-term findings and assess adaptation, persistence, and microbiota stability; iii) impact of feed additives on product quality; iv) economic feasibility, especially for rural and commercial systems. Only through such a pragmatic approach can the sector ensure sustainable development through scientifically sound, economically viable, and practically implementable mitigation strategies.

DATA AVAILABILITY STATEMENT

No new data were produced.

CONFLICT OF INTEREST STATEMENT

All authors declare that they have no competing interests.

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