Developing safe and effective enteric methane mitigation solutions for dairy cattle: Gaps in knowledge we cannot ignore

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Introduction

We are in the midst of an agricultural renaissance defined by global innovation in the livestock sector to meet the demand for meat and milk in the 21st century. The catalyst was the Global Methane Pledge, established in 2021, which calls for a 30% reduction in anthropogenic methane emissions to avoid reaching a 1.5°C increase in global warming by 2030. It didn't take long for pundits including academics, policy makers, and advocates to call for the depopulation of cattle on our plant and the transition to plant-based diets. In parallel, a tidal wave of vehement focus on livestock methane from non-government organizations (NGOs), philanthropic organizations, and venture capitalist firms rose with unprecedented organization. In December of 2023, the Dairy Methane Alliance was signed by Nestlé, Danone, General Mills, Kraft Heinz, Bel Group, and Lactalis at the United Nations Climate Change Conference in Dubai, United Arab Emirates. The dairy food manufacturers, with support from the Environmental Defense Fund, have committed to disclose annual emissions from their dairy supply chains and develop and execute a plan to reduce methane emissions. A plethora of eager startup companies focused on bold but undeveloped ideas including anti-methanogenic vaccines (e.g., Arkeabio), seaweed (e.g., Symbrosia, Rumin8, and Alga Biosciences), and genetically-engineered crops (e.g., Biolumic), and methane traps and biosensors (e.g., ZELP) aim for prominence in a crowded field of aspiring agricultural biotech gamechangers. Applying pressure for livestock methane mitigation solutions are government mandates to reduce livestock methane and emerging carbon crediting programs such as the Verified Carbon Standard Program. The justified hysteria surrounding livestock methane has demanded the discovery, approval and adoption of enteric methane mitigation technologies; albeit, media excitement around a “silver bullet” solution and lack of education and transparency surrounding innovation are outpacing the holistic testing required to prove safety and efficacy of enteric methane mitigation solutions.

The current state of dietary methane-mitigation solutions is summarized by Honan and coworkers (2021). These include 3-nitrooxypropanol (3NOP; a direct inhibitor), fatty acids (i.e., medium-chain and unsaturated; a rumen modifier), plant-extracts (e.g., essential oils), tannins, saponins, ionophores, or nitrate with methane-reducing efficacy ranging from 5 to 30%. In stark contrast, feeding red seaweed, such as bromoform-containing *Asparagopsis taxiformis* and *Asparagopsis armata,* has been demonstrated to reduce methane yield by as much as 97% in cattle (Lean et al., 2021). The mode of action involves the unique presence of halogenated compounds, such as bromoform, in these seaweed species. Halogenated methane analogs inhibit methane production by binding and sequestering the prosthetic group required by methyl-coenzyme M reductase.

Funding agencies are accelerating programs focused on the study of methane-reducing feed additives such as the Greener Cattle Initiative of the Foundation for Food and Agricultural Research and the California Department of Food and Agriculture Livestock Enteric Methane Emission Reduction Research Program. Moreover, international teams are forming to align with NGO directives focused on enteric methane solutions. This brief perspective aims to highlight select knowledge gaps surrounding feed additives and enteric methane mitigation.

Energetics and nutrient use

In cattle, enteric methane production represents a loss of 2 to 12% of gross energy intake (6.5 to 7% mean; Johnson and Johnson, 1995). However, we lack adequate perspective regarding the effects of methane-reducing feed additives (e.g., *Asparagopsis taxiformis* [i.e., seaweed] or bromoform) on energy partitioning in cattle. We must consider changes in gross, digestibility, gaseous, urinary, fecal, metabolizable, maintenance, tissue, and milk energies to adequately characterize and model the effects of these dietary approaches on energy utilization. Moreover, we must consider how energetics of methane reduction is influenced by plane of nutrition and energy balance since nutrient prioritization for growth and lactation are not constant. The scientific community also lacks a firm understanding of the effects of feed additives on nutrient flow to the lower gut. For instance, studies are needed to study the rumen pool sizes and omasal flows of nutrients to determine digestion parameters including fractional rates of carbohydrate digestion, and microbial growth and yield of microbial biomass in response to methane reduction by feed additives.

Human food composition and safety

Dietary ingredients that inhibit enteric methane emissions have potential to impact the composition and sensory properties of dairy foods. Residues of human food safety concern deserve special attention. Synthetic bromoform, or bromoform within seaweed algae, is a probable human carcinogen (EPA, 2018). A limit of 80 µg/L bromoform in drinking water has been established by the United States Environmental Protection Agency (EPA, 2018). Current evidence suggests that bromoform is able to transfer from animal feed to milk from cows but not meat (Muizelaar et al., 2021); however, transfer was not observed in all animals and feeding level is likely to influence milk bromoform (or bromide) enrichment. We lack an understanding of ruminal bromoform degradation (i.e., reductive dehalogenation) and bromoform enrichment in organs to adequately define safety. Minerals including iodine and heavy metals (i.e., arsenic) are also a potential concern. Dietary supplementation of *Asparagopsis taxiformis* has been shown to increase milk iodine concentrations (Stefenoni et al., 2021). Iodine excess has potential to trigger thyroid dysfunction in humans including individuals with preexisting thyroid disease (Southern and Jwayyed, 2023). But we lack an understanding of how high-temperature pasteurization influences milk concentrations of volatile halogenated compounds. We also need to consider how feed additives that modify rumen fermentation or intestinal nutrient digestibility influence the macronutrient composition of milk. For instance, monensin feeding has been shown to modify milk fat composition (Duffield et al., 2008). Such efforts should also focus on whether methane-reducing feed additives influence the physical and sensory properties of dairy foods.

Animal health and safety

Research that examines the impact of methane-reducing feed additives on animal health is needed to ensure safety for the animal. One concern is whether the additive decreases voluntary intake, which has potential to exacerbate the magnitude of negative energy balance during early lactation and potentially increase the incidence of metabolic disease during the periparturient period. Dietary approaches that inhibit methane but compromise fiber digestibility (e.g., tannins [de Oliveira et al., 2007]), rumen fermentation e.g., iodoform [Thorsteinsson et al., 2023]), or palatability (e.g., bromoform-containing seaweed [Wasson et al., 2023]) to inhibit energy intake may enhance milk production efficiency in healthy animals but may predispose animals to poor health outcomes near parturition. Feeding *Asparagopsis taxiformis* at 67 grams of dry matter per day (1X target dose) caused papillary necrosis and inflammation of the rumen epithelium (Muizelaar et al., 2021); however, we can expect that feeding level and source of seaweed as well as the feeding management protocol may influence this outcome. To overcome observed decreases in voluntary feed intake with seaweed or iodoform feeding, or perhaps an alternative additive, the use of agents with preferred tastes and aromas, such as citrus, garlic or molasses, may overcome palatability issues. Therefore, manufacturers are likely to market co-additive technologies to achieve methane reduction without compromised feed intake (e.g., Enterix from Mootral [iodoform plus garlic; Abertillery, UK]). For current approval by the United States Food and Drug Administration, future research will need to focus on how methane-reducing feed additives impact measures of animal health (via urinalysis, blood chemistry and hemogram, and necropsy) under different feeding levels (i.e., 1x, 2x, 5x target dose) or physiological states (pregnancy, or early or late lactation) following extended duration of the intervention (i.e., 3 months).

Feeding duration and adaptation

Each methane-reducing technology is likely to inhibit ruminal methanogensis differently over time. Direct inhibitors will inhibit methane production sooner than ruminal modifiers. For instance, maximum reductions in methane production are observed approximately 6 hours post-feeding for 3NOP (Hegarty et al., 2021), which means that such a technology is best suited for confinement dairy production systems with consistent feed management unless alternative delivery systems are employed (e.g., water enrichment or bolus). Rumen modifiers that require shifts in bacterial populations are expected to require more time to elicit an inhibitory outcome on methanogenesis (e.g., monensin).

We must also consider the interactions between feed additives and the nutrient composition of the diet. Feedings cows higher starch and lower fiber diets will lower methane production, relative to lower starch and higher fiber diets (Schilde et al., 2021). Schilde and coworkers (2021) demonstrated that methane reduction with 3NOP was more pronounced in cows fed higher starch diets. Indeed, Kebreab et al. (2023) demonstrated that methane reduction for 3NOP-supplemented cows was approximately 31 to 33% across 14 experiments; however, the range was 15 to 65%. The meta-analysis concluded that increases in dietary neutral detergent fiber and fat content reduces efficacy of 3NOP. Apparent microbial adaptation was also less pronounced in cows fed higher starch diets but still evident (i.e., cows fed 3NOP and low starch diets had methane emissions that returned to baseline after 20 wk of 3NOP supplementation). The methane mitigation effect of *Asparagopsis taxiformis* also appears to be transient (Wasson et al., 2023). Maximum methane reductions were observed within the first month (i.e., 49%) but faded to 14% by the end of the second month. Such observations suggest microbial adaptation; however, we must also consider bromoform instability as a potential explanation. Manufacturers are considering alternative methods of preparing and storing *Asparagopsis taxiformis* to prevent volatilization and extend shelf-life (e.g., oil coating). Feed additive parallel usage and sequencing (i.e., alternating additives) protocols, including early life interventions, are being considered to ensure methane reduction over the lifespan of animal.

Methane sensor technologies and protocols of use require scrutiny

Measurement, reporting and verification (MRV) programs require establishing baseline enteric methane emissions and methane emission reduction factors. The accurate measurement of absolute methane emissions at the individual cow is needed. Unfortunately, on-farm methane measurement technologies are still in their infancy. The use of the GreenFeed system (C-Lock Inc., Rapid City, South Dakota) has gained momentum; however, such technology has limitations. Specifically, the system provides estimates of total daily methane emissions derived from limited spot sampling, typically less than 20 minutes per day. Recent findings suggest poor agreement for methane production data derived from cows evaluated using the gold-standard respiration chambers and the GreenFeed system (Bayat et al., 2023). We also need to consider protocols of use for methane sensor technologies, which may influence feeding behavior and time of measurement relative to feeding. Although my lab utilizes GreenFeed, we do not claim absolute measurements but report estimates and relative efficacy values until suitable correction factors are developed.

Enhanced efficiency to reduce methane intensity requires prioritization in Global South

The demand for animal protein in Asia and Africa is expected to continue to increase (FAO, 2023); however, the smallholder dairy production system of the Global South is characterized by high enteric methane emissions per unit of milk produced. This said, a high level of variation in emission intensities has been observed across smallholder farmers in Kenya (e.g., 20 to >1,000 CO_2 equivalent/kg of crude protein; Ndung'u et al., 2022). Selective breeding, enhanced animal health, and the use of ration balancing are a means to improve efficiency. For example, India has more than 300 million cattle and buffaloes managed by approximately 75 million farmers. Feeding balanced diets as part of India's National Dairy Development Board Rational Balancing Programme on the basis of total digestible nutrients and crude protein resulted in a decrease in dry matter intake and enteric methane emissions by more than 13% (FAO, 2023). Feeding costs were reduced 9%. Reducing the prevalence of disease should also be considered as a methane mitigation strategy. In the United Kingdom, Skuce (2022) estimates a 10% reduction in greenhouse gas emissions with the improvement in animal health; albeit, the potential for greater impact is expected for low and middle-income countries. The use of feed additives or improved veterinary care will require an economic and distribution model that works within the constraints of a smallholder production system.

Conclusion

Feed additives are promising strategy to reduce enteric methane production; however, we need to address critical gaps in knowledge to ensure benefit for cows, humans, and our environment. We must be careful not to portray methane-reducing outcomes as standard norms considering that feed management is influential. We need to expedite research that addresses concerns related to microbial adaptation, and animal and human food safety to ensure farmer and consumer confidence in these technologies. We must also advocate for strict method standards when measuring enteric methane emissions. Efforts must also be made to close the gap in production efficiency between large-scale and smallholder production systems.

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