

Mitigation of Enteric Methane Emissions: A Down-to-Earth Perspective

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Introduction

Methane is an anthropogenic greenhouse gas (GHG) and potent climate pollutant that has contributed ~0.5°C to observed global warming from the years 1850-1900 to 2010-2019 (IPCC, 2021). Although cattle are part of a natural biogenic carbon cycle involving carbon recycling between animals and plants, livestock are estimated to contribute ~30% of global anthropogenic methane emissions (Tian et al., 2016; Jackson et al., 2020; Saunio et al., 2020). The additional public concern is that the global livestock population has tripled over the past century; which is expected to expand in the future (Godfray et al., 2018; Henchion et al., 2021). Global animal protein supply is anticipated to increase 17% from the year 2017 to 2050 under a “business as usual” scenario (Henchion et al., 2021). Therefore, methane emissions from livestock are expected to increase. Attention has centered on the development and application of dietary feed additives that act as rumen environment modifiers (e.g., essential oils) or direct inhibitors (e.g., 3-nitroxypropanol [3-NOP] or halogen-containing seaweed) of enteric methane production. Dietary approaches to inhibit ruminal methanogenesis have strong scientific merit to reduce methane emissions from cattle and slow the progression of climate change in the short-term; however, limited evidence supports the ability of any single approach to enhance the energetic efficiency of milk production in a consistent manner with a clear mode of action.

Although feed additives to reduce enteric methane production in ruminants are exciting to consider, we cannot ignore the reality that a large disparity exists between developed and developing countries with regard to methane emissions and production efficiency. Zhang and coworkers (2022) provided an analysis of a 130-year global inventory of methane emissions from livestock. In the 1890s, developed and developing countries accounted for 44% and 56% of total methane emissions ($\text{Tg CH}_4 \text{ yr}^{-1}$), respectively. In the 2010s, 18% and 82% of global methane emissions were derived from developed and developing regions, respectively. The greatest increases in methane emissions between the 2010s and 1890s were in South Asia (29% of total), Brazil (12%), Northern Africa (12%), and China (11%). This is problematic when we consider that South Asia is expected to experience a 64% increase in energy-corrected milk production by 2030, relative to levels observed in 2017 (Henchion et al., 2021). At the country level, India was the top emitter in the 2010s at $24.0 \text{ Tg CH}_4 \text{ yr}^{-1}$, as compared to the United States of America at $8.3 \text{ Tg CH}_4 \text{ yr}^{-1}$. From a dairy perspective, milk production efficiency in cattle (or buffaloes) in the developing world pales in comparison to developed nations. This proceeding will examine the energetics and efficiency of milk production in cattle including a focus on feed ingredients that can substantially inhibit enteric methane production. A case study of India will explore the

challenges and potential solutions that require immediate consideration to enhance milk production efficiency and reduce the intensity of methane emissions.

Energetics of methane and milk production

The 8th Revised Edition of the Nutrient Requirements of Dairy Cattle summarizes the classical energy flow system (NASEM, 2021). Gross energy (GE), or the amount of energy in the feed, minus fecal energy is digestible energy (DE). Digestible energy is divided into six fractions: digested neutral detergent fiber [NDF], digested starch, digested fatty acid, digested residual organic matter (i.e., sugars, pectins, gums, glycerol of triacylglycerol, and fermentation acids), supplemental nonprotein nitrogen on a crude-protein equivalent basis, and digested crude protein. Energy lost in urine and via methane is subtracted from DE to obtain ME. Urinary energy can be estimated from urinary N excretion. Gaseous energy is a function of dry matter intake (DMI) and the content of fatty acids and digested NDF in the diet. Net energy is ME minus heat production from digestive and metabolic processes (i.e., heat increment). The conversion of ME to net energy of lactation is 0.66.

The use of indirect calorimetry has demonstrated that methane losses vary from 2 to nearly 12% of GE intake (Johnson et al., 1993). The average loss being ~5 to 6%. Johnson and Johnson (1995) stated that as diet digestibility increases, variability in methane loss also increases. The authors describe two primary modes of action that cause variability in methane production by cattle. First, the amount and type of dietary carbohydrate fermented in the reticulorumen, which involves a balance between rate of carbohydrate fermentation and passage of feed. Second, the regulation of propionate production (a hydrogen sink), as opposed to acetate, regulates hydrogen supply and methane production. The authors further postulated that if the acetate to propionate ratio was 0.5, the loss of substrate energy as methane would be 0%; however, Wolin and Miller (1998) suggested that if all carbohydrates were fermented to acetate, and none to propionate, then methane energy loss would be 33%. This is significant when we consider that the acetate to propionate ratio in rumen fluid can range from less than 1:1 to greater than 4:1 in low and high forage diets, respectively (Russell, 1998).

In a retrospective analysis of data derived from 20 energy metabolism studies involving 579 lactating dairy cows, Yan and coworkers (2010) evaluated methane energy output in relationship to factors that define energetic efficiency. Methane energy per unit of GE intake and methane energy per unit of milk energy output (i.e., emissions intensity) were lowered with increasing feeding level (ME intake/ME requirement for maintenance), milk energy per unit of metabolic body weight ($\text{kg}^{0.75}$), as well as intakes of GE, DE, or ME per unit of metabolic body weight. The authors estimated that methane energy is 8.5% of GE intake at maintenance feeding level but can decrease by half if feeding level is high. Moreover, a reduction of energy expenditure on maintenance as a proportion of ME intake from 100 to 40% could decrease methane energy from 7.6 to 3.6%, respectively. Indeed, high-yielding cows produce less methane per unit of milk energy because of “dilution of maintenance”. Gains of 100 kg of milk per lactation are predicted to result in a 7.3% decrease in methane per unit of energy-

corrected milk at a production level of 7,000 kg; which is in contrast to 3.1% for cows producing 13,000 kg without changes in rumen fermentation or nutrient digestibility (Kebreab et al., 2008; Knapp et al., 2014). Feeding high-quality (i.e., more energy-dense and digestible) to cattle that have a higher genetic merit for milk production, and better management, have the confirmed potential to reduce methane energy output as a proportion of GE intake and dilute the cost of maintenance.

Approaches to reduce conversion of gross energy to methane energy

It is often argued that the inhibition of methanogenesis in the ruminant has the potential to enhance milk production. Reducing the conversion of GE to gaseous energy could enhance the conversion of GE to DE and ME. As described by Beauchemin and colleagues (2020), cows fed diets with 70% digestible energy, a moderate decrease (e.g., 25%) may only increase ME by 0.75% to 4.25%. Because the efficiency of converting ME to net energy for lactation is 0.66, it may be difficult to observe improvements in milk production (especially in small population sizes often used for scientific research). The authors state that more severe inhibition of methane production beyond 50% without compromised DMI or digestibility may be required to observe substantial increases in milk production. Two dietary feed ingredients that have potential to inhibit methanogenesis 30% or more include 3-NOP or halogen-containing seaweed.

3-nitrooxypropanol (3-NOP)

The competitive inhibitor 3-NOP reduces enteric methane production by 20 to 80% in beef cattle, dairy cattle, and sheep (average of 30%; Martínez-Fernández et al., 2014; Hristov et al., 2015; Lopes et al., 2016; Vyas et al., 2016). As a structural analog of methyl coenzyme M, 3-NOP blocks the active site of methyl-coenzyme M reductase, which inhibits the last step of methanogenesis (Duin et al., 2016). A plethora of studies have assessed the impacts of 3-NOP on methane and hydrogen production, milk production and composition, DMI, feed efficiency, methanogen growth, digestibility, and energetics (Jayanegara et al., 2018; Almeida et al., 2021).

The effects of 3-NOP on methane and hydrogen emissions are consistent. In a 12-week study of lactating dairy cows, Hristov and colleagues (2015) reported a 30% reduction in methane production (assessed using GreenFeed system [C-Lock Inc., Rapid City, SD]) when 3-NOP was included in the ration at 40 to 80 mg/kg of dry matter [DM]. Melgar and colleagues (2021) also used a GreenFeed system to measure enteric methane production in dairy cows fed 3-NOP (60 mg/kg of DM) for 15 weeks. They observed that 3-NOP reduced emission yield and intensity by 27% and 29%, respectively. van Gastelen and coworkers (2020) used respiration chambers to measure enteric methane production in early lactation dairy cows fed 3-NOP (51 mg/kg of DM) for 16 weeks. In this study, 3-NOP reduced methane emission at 55 and 111 days in milk by an average of 18.5%. The observed decrease in methane production develops with a consistent increase in H₂ emissions. Normal production of methane by methanogenic archaea involves reduction of CO₂ to CH₄ with H₂. Increases in ruminal H₂ concentrations has potential to lead to the down-regulation of H₂-generating

pathways and up-regulation of H₂-consuming pathways. An increase in H₂ emissions with 3-NOP treatment has been observed by Melgar et al. (2021) and van Gastelen et al. (2022). These collective changes on CH₄ and H₂ emissions by 3-NOP have been confirmed by recent meta-analyses (Jayanegara et al., 2018; Almeida et al., 2021).

There have been a number of recent studies examining the interactions of 3-NOP feeding with other dietary ingredients to identify strategies to maximize methane inhibition. van Gastelen and coworkers (2022) concluded that 3-NOP inhibited methanogenesis (and increased H₂ emissions) more when lactating cows were supplemented with corn silage as compared with cows fed grass silage. Schilde and coworkers (2021) confirmed that feeding high concentrates in combination with 3-NOP synergistically lowered methane yield, more than low concentrate diets containing 3-NOP. Feeding beef cattle a high-forage diet (90% barley silage) containing 3-NOP (200 mg/kg of DM) and canola oil (50 g/kg of DM) suppressed ruminal methanogenesis more than when either were offered alone (Gruninger et al., 2022). The authors concluded that 3-NOP inhibited the hydrogenotrophic methanogenesis pathway, whereas oil caused changes in the rumen microbial community to alter rumen fermentation. The addition of monensin to beef cattle rations supplemented with 3-NOP was unable to lower methane yield more than 3-NOP alone (Vyas et al., 2018). It is conceivable that co-supplementation strategies involving 3-NOP and alternative inhibitors of methanogenesis (with different modes of action) will be required to inhibit methane production more than 50% and thus favorably impact milk production in dairy cattle.

The effects of methane inhibition by 3-NOP on milk production and composition, DMI, feed efficiency, and bodyweight have received attention. van Gastelen and colleagues (2020) found no effect on any of these variables in early lactation dairy cows fed 3-NOP (51 mg/kg of DM) for 16 weeks. These findings are consistent with Reynolds and colleagues (2014), who found no change in DMI, yields of milk or fat-corrected milk, or milk energy in lactating dairy cows administered 3-NOP (500 or 2,500 mg/d delivered into the rumen via fistula; 2x daily before feeding) for 5 weeks. Similarly, Melgar and coworkers (2021), as well as Lopes and colleagues (2016), observed no changes in DMI or milk yield in lactating dairy cows, except for an increase in milk fat concentration with 3-NOP feeding. These findings are consistent with the results from Hristov and colleagues (2015), who found that neither DMI or milk production were affected by 3-NOP supplementation (40 to 80 mg/kg feed DM) in lactating cows supplemented for 12 weeks; however, milk protein and lactose yields, as well as bodyweights were increased by 3-NOP treatment. They also observed that methane emission per unit of DMI or per unit of energy-corrected milk were about 30% less for the cows treated with 3-NOP, relative to unsupplemented cows. The increase in bodyweight was also observed in mid-lactation dairy cows administered 2,500 mg of 3-NOP per day for 28 days with a 38% forage diet, which occurred without changes in DMI or milk production (Haisan et al., 2014 and 2017). The ability of 3-NOP to reduce energy lost as methane appears to spare energy to support milk component synthesis or body tissue accretion; albeit, the impact of 3-NOP on nutrient utilization and partitioning is likely influenced by stage of lactation and the homeorhetic mechanisms of the cow.

An investigation by van Gastelen and colleagues (2020) observed that 3-NOP promoted positive effects on total-tract digestibility of nutrients, including a greater ME intake to GE intake ratio. An improved total tract digestibility with 3-NOP feeding has been observed in other studies (Hristov et al., 2015; Haisan et al., 2017; Melgar et al., 2020). van Gastelen and colleagues (2020) hypothesized that improved digestibility may result in more efficient rumen fermentation due to greater availability of propionate, relative to acetate. A decrease in the ratio of acetate to propionate in response to 3-NOP consistently develops with 3-NOP treatment (Haisan et al., 2014; Martínez-Fernández et al., 2014; Romero-Perez et al., 2014; Romero-Perez et al., 2015; Lopes et al., 2016; Haisan et al., 2017; Martínez-Fernández et al., 2018; van Gastelen et al., 2020). Greater propionate availability in response to 3-NOP may also explain frequently observed increases in bodyweight. Propionate is the main glucogenic precursor in ruminants, and promotes the release of insulin, which promotes fat storage and reduces mobilization of body reserves (van Knegsel et al., 2007). The observed increase in H₂ emissions with 3-NOP treatment does not appear to negatively impact rumen function. This could be a potential concern since an increase in H₂ partial pressure in the rumen is known to cause negative feedback on rumen fermentation, feed intake or digestibility (Leng, 2014). This said, 3-NOP treatment has been shown to increase fecal nitrogen excretion and decrease nitrogen digestibility to decrease body nitrogen balance (Reynolds et al., 2014), which should be examined further considering nitrous oxide emissions from manure is another concern for climate change.

Seaweed

In coastal regions, seaweeds have been a part of livestock diets since initial agricultural practices began (Heuzé et al., 2017). Members of the red micro algae genus *Asparagopsis*, particularly *A. taxiformis* and *A. aramata*, have gained considerable attention because of their ability to inhibit enteric methanogenesis (Machado et al., 2014). These seaweeds contain a high abundance of bioactive compounds, called halogenated methane analogues (HMAs), that inhibit the activity of methanogens. Examples of these HMAs include bromochloromethane (Machado et al., 2014; Heuzé et al., 2017; Stefenoni et al., 2021), bromoform (Brooke et al., 2020), chloroform (Abbott et al., 2020), and dichloromethane (de al Moneda et al., 2019). Their specific mode of action is to bind with reduced vitamin B₁₂, blocking the cobamide-dependent methyltransferase reaction required for formation of methyl-coenzyme M (Wood et al., 1968). In *Asparagopsis*, the most abundant bioactive constituent and most important contributor to its antimethanogenic activity is bromoform, followed closely by dibromochloromethane (Paul et al., 2006).

Dietary supplementation with *A. taxiformis*, *A. aramata*, or isolated HMAs (i.e., bromochloromethane or chloroform) consistently reduces methane emissions in sheep (Li et al., 2016), goats (Mitsumori et al., 2012), and cattle (Johnson et al., 1972; Roque et al., 2019; Kinley et al., 2020; Roque et al., 2021; Stefenoni et al., 2021). Johnson and colleagues (1972) observed a complete inhibition of methane production (100%) in steers administered 5.5 g of bromochloromethane per d for 28 days. Kinley and colleagues (2020) observed a 40% and 98% reduction in enteric methane production

(assessed by respiration chambers) in steers supplemented with *A. taxiformis* at 0.10% and 0.20% of organic matter, respectively, for 90 d. In lactating dairy cows administered *A. aramata* at 0.5% or 1% of organic matter for 21 d, methane production (assessed using the GreenFeed system) was reduced by 26% and 67%, respectively (Roque et al., 2019). Similarly, Stefenoni and coworkers (2021) observed a 34% reduction in methane emissions (assessed using the GreenFeed system) in lactating dairy cows supplemented with *A. taxiformis* at 0.5% of DM for 28 d. Supplementing lactating goats with 0.66 mg/kg BW bromochloromethane per d for 70 d resulted in a 32% reduction in methane emissions, which was assessed using respiration chambers (Abecia et al., 2012). Across studies, the reduction in methane emissions by red microalgae or its bioactive compounds consistently increases H₂ production (Kinley et al., 2020; Roque et al., 2019 and 2021; Stefenoni et al., 2021).

The effect of reduced methane emissions by seaweed on productivity (i.e., bodyweight gain or milk production) varies greatly. The reduction in methane emission observed by Kinley and colleagues (2020) in steers was accompanied by weight gain increases of 53% and 42% for the 0.10% and 0.20% of organic matter inclusion of *A. taxiformis*, respectively, with no changes in DMI or feed conversion efficiency. Although Johnson and colleagues (1972) observed a complete inhibition of methane production by bromochloromethane, only a numerical increase in average daily gain was observed (i.e., not statistically significant). Roque and coworkers (2019) observed a 12% reduction in milk yield, and lower DMI, in lactating cows administered *A. aramata* at 1% of organic matter for 21 d. Similarly, Stefanoni and colleagues (2021) observed a 6.5% reduction in DMI, milk yield, and energy-corrected milk yield in lactating dairy cows supplemented with *A. taxiformis* at 0.5% of DM for 28 d. In contrast, Abecia and colleagues (2012) observed a 36% increase in milk yield in lactating goats.

While digestibility and energetics data are limited in studies of seaweed feeding to cows, Johnson and coworkers (1972) found no effect of bromochloromethane on the digestibility of DM, energy, or acid detergent fiber in steers. Reducing methane emissions by supplementing *A. taxiformis*, *A. aramata*, or isolated HMAs consistently results in a decrease in the proportion of acetate to propionate in the rumen (Abecia et al., 2012; Roque et al., 2019; Kinley et al., 2020; Roque et al., 2021; Stefenoni et al., 2021). Safety concerns do exist for seaweed feeding. Milk iodine concentrations are elevated in cows fed a seaweed mix of *Ascophyllum nodosum* and *Laminaria digitate* (Newton et al., 2021). Although bromoform does not appear to accumulate in tissue, the compound does appear to be excreted in urine and milk (Muizelaar et al., 2021). Signs of inflammation, hemorrhages and ulcers have also been documented following the histological examination of the rumen wall and papillae of cows fed *Asparagopsis taxiformis* (Muizelaar et al., 2021). These findings are concerning and further testing should be required before seaweed feeding is adopted as farm practice. Such an effort will help be beneficial to ensure consumer acceptance of the technology if proven safe.

Why we must prioritize enhancing feed efficiency in developing nations: A case study of India

Developing nations such as India are major contributors to global anthropogenic emissions of GHG. In recent years, the share of Indian agriculture was 7% of global emissions (CO₂ equivalent [CO₂e]) from agriculture (Pathak, 2015). By 2050, the number of milk-consuming households in India is projected to increase from 185 million to 349 million (Gupta and Dasgupta, 2020). It is logical that India is the world's largest producer of milk, producing 195 million metric tons in 2020 or ~22% of global production (FAO, 2020). The milk produced in India contributes to a major portion of the gross income of rural households and most of the livestock sector gross domestic product. In stark contrast to North America and Europe, low-producing buffaloes (*Bubalus bubalis*) yield more than half of the milk in India, followed by indigenous cows (*Bos indicus*) and indigenous cows crossbred with exotics (e.g., Holstein, Jersey, etc...). It is estimated that India currently has over 50 million dairy cows or ~18% of the world's total population (Steenland, 2019). Moreover, countries of the Indian subcontinent manage 37% of the world's dairy goats and produce 41% of the world's goat milk (Pulina et al., 2018). The vast population of ruminants in India is a concern because enteric production of methane by ruminants is the largest anthropogenic source in agriculture; therefore, in India, as the demand for and production of milk rises, we can expect an increase in GHG emissions from ruminants.

The milk revolution, often referred to as the White Revolution, was key to increase milk production by 400% from 1968-1969 to 2003-2004 (Deka et al., 2015). Operation Flood was a government-sponsored program that promoted crossbreeding, improved access to feed and veterinary services, and enhanced markets, and milk processing and preservation infrastructure to avoid chronic milk shortage in India. The program augmented rural incomes and provided milk and dairy products at fair prices for the consumer. Today, Indian farming primarily consists of traditional smallholder production systems managing 1 to 5 animals that produce approximately 5 liters of milk per day; albeit, the number of larger commercial systems is gradually increasing. In an evaluation of cattle production in Eastern India (Gupta et al., 2014), the majority of farmers were unable to spare land for fodder production, followed their own feeding practices, were unsatisfied with milk production levels, required training in diet formulation, and desired a transition from natural breeding to artificial insemination to increase the population of crossbred animals with higher milk production and heat tolerance.

Although the White Revolution was a success for Indian agriculture, the country is faced with an agrifood challenge in an era of climate change. Milk and dairy products are a major source of affordable and nutritious food for millions of Indians (Ohlan, 2012). For Indians that consume animal-sourced foods, milk provides the highest proportion of total caloric intake. This is especially important for growing infants and lactating mothers. Following UN Population Division forecasts, the human population in India is expected to increase 194 million between 2015 and 2030 (Liu et al., 2018). This is the highest rate of growth in Silk Road Economic Belt and Maritime Silk Road

countries, and it is anticipated that India will surpass China as the most populous in the world by 2030 (Liu et al., 2018). In parallel, the FAO estimates per capita consumption of meat and milk in India will increase 94% between 2006 and 2050 (highest increase in the world; Searchinger et al., 2018). Indeed, an increase in dairy imports has potential to offset demand for domestic production. Total food imports including dairy are expected to increase 8.5 to 18.3% by 2050 (Hamshere et al., 2014); but, these projections will be influenced by government policies toward animal agriculture. Domestic milk production will need to increase to meet projected demand. Despite producing the most milk of any country in the world, milk productivity in India remains one of the lowest (Bardhan and Sharma, 2013). Such poor efficiency is unlikely to meet the future demand for milk. This is especially concerning when we consider that India ranks 97 out of 118 on the Global Hunger Index and 39% of children under five in India are defined as 'stunted' due to malnutrition (of below average height; von Grebmer et al., 2016; Ritchie et al., 2018).

In India, it is estimated that 90% of total methane emissions from enteric fermentation are contributed by buffalo and cattle and the remainder from small ruminants (e.g., goats) and other domestic animals (Swamy and Bhattacharya, 2006). In 2006, approximately 48% and 35% of enteric and manure methane emissions were derived from indigenous cattle and buffaloes, respectively. Enteric and manure methane density (Gg/sq. km/y) is highest in Northern states such as Bihar, Uttar Pradesh, Rajasthan, and Punjab. As compared to dairy production in North America or Europe, methane yields per kg of protein produced by dairy ruminants are greater in South Asia countries including India (Chang et al., 2021). Crossbreeding taurine and indicine cattle has potential to increase milk production efficiency. Across all Indian states and territories, mean GHG intensity per unit of milk for crossbred cows is 1.21 kg of CO₂e kg⁻¹ milk versus 2.96 kg of CO₂e kg⁻¹ milk for indigenous cows (Patra, 2017). For perspective, the average enteric methane emissions intensity in the United States is ~0.25 kg of CO₂e kg⁻¹ milk (Tricarico et al., 2020). A similar situation is observed when evaluating emissions on the basis of milk energy. For example, mean intensity for crossbred cows is 0.41 kg of CO₂e MJ⁻¹ milk energy versus 1.00 kg of CO₂e MJ⁻¹ milk energy for indigenous cows. Crossbred cows have lower GHG intensity per unit of milk when compared to buffaloes (1.21 versus 1.85 kg of CO₂e kg⁻¹ milk); albeit, intensity of GHG emissions is comparable on the basis of milk energy. Enhanced regional utilization of agro-industrial byproducts, feeding nutrient-balanced diets, and accelerated adoption of artificial insemination and crossbreeding efforts are promising approaches to enhance efficiency and reduce methane emissions from cattle; however, such strategies are needed within the framework of existing religious and socio-economic challenges with government support.

Balanced ration formulation is a means to increase milk production efficiency in India. The FAO (2012) suggests that balanced ration formulation can increase daily income from rearing livestock by ~10% in India. Blümmel and coworkers (2009) estimated that milk yield per animal in India can increase from 3.6 to 9 L/d by feeding cows nutrient-balanced diets. Moreover, increasing milk yield per animal from 3.6 to 12 L/d would reduce the number of livestock by 70%, feed required by 48%, and methane

production by 46%; albeit, this is dependent upon increasing the energy density of diets in a country with limited availability of concentrates and poor quality fodder. Goswami and coworkers (2013) demonstrated the feasibility of reducing feed cost by 19% compared to a routine feeding plan when formulating diets for crossbred dairy cows yielding 5 to 10 kg of milk/d in central India. Therefore, advanced ruminant nutrition will enhance the efficiency of nutrient use for milk production in India, which will decrease nutrient requirements for maintenance, GHG emissions, and ruminant animals required per unit of milk or milk energy.

Summary and Future Directions

The use of feed additives such as 3-NOP or seaweed to inhibit ruminal methanogenesis has merit; however, we must temper enthusiasm, and be rational and transparent. For 3-NOP, we require studies that investigate the use of 3-NOP over full lactations, assess various modes of delivery to ensure that cattle in non-confinement management scenarios (e.g., grazing systems) also benefit, and assess its impact on nutrient flow to the duodenum. It is also apparent that the magnitude of efficacy for 3-NOP is highly influenced by the diet. We must continue to examine the interaction of 3-NOP with dietary ingredients that influence rumen fermentation and methanogen activity. For seaweed, we must be confident that this approach won't increase the presence of iodine, bromine, arsenic, and other halogenated compounds in meat or milk to limits of human safety concern. We need to carefully consider how long-term feeding of seaweed impacts the health and productive lifespan of the animal, examine stability, bioavailability, and safety of seaweed compounds, and require complete life cycle assessments that consider the production, processing, transport, and use of the product to ensure that the net impact of the technology on our environment is positive and economically competitive. We must also consider how methane inhibitors influence the emissions of other GHG from the rumen or manure including nitrous oxide, better understand how rumen ecology adapts (or doesn't), examine compound replacement or additivity on methane emissions, consider early-life methane-inhibitor interventions that have long-term benefit, and better define energetic conversion of GE to milk energy. We must also aggressively challenge and acknowledge the limitations of the scientific methods that we utilize to define efficacy of methanogenesis inhibitors.

Feed additives that inhibit methanogenesis may not be cost-effective or practical in developing nations with unique production systems. It is highly likely that technologies that inhibit ruminal methanogenesis, if approved for use, are more likely to be adopted in developed regions of the world. Therefore, the use of dietary approaches to lower enteric methane production will need to be high in order to enhance the conversion of gross energy to milk (and not methane) but also compensate for cattle that don't receive such interventions on a global scale. This is why we must continue to enhance the productive efficiency of cattle (and buffaloes) in regions of the world that are far-behind North America and Europe.

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