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REPORT 5A - NEAR-TERM METHANE REDUCTION OPTIONS:

Opportunities & Challenges for Reducing Enteric Methane from Alberta Beef and Dairy Production

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REPORT 5A - NEAR TERM METHANE REDUCTION OPTIONS: OPPORTUNITIES & CHALLENGES FOR REDUCING ENTERIC METHANE FROM ALBERTA BEEF AND DAIRY

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ABSTRACT

The reduction of greenhouse gas emissions is a global goal across sectors. Methane, an especially potent greenhouse gas relative to carbon dioxide, is the target of the Global Methane Pledge, an undertaking by over 100 countries to reduce methane emissions by 30 percent by 2030. The agricultural sector is uniquely positioned to support Canadian methane reductions through mitigation of enteric fermentation in cattle. Enteric fermentation in dairy and beef cattle contribute to over 85 percent of methane emissions from the agriculture sector. Different mitigation strategies and technologies have demonstrated variable effect on methane reduction, depending on factors related to cattle diet, management, and operational practices. Relevant research and literature based on criteria related to potential application in western Canada and Canadian cattle production in the beef are dairy sector was collected and reviewed using PRISMA approach. Research in the beef and dairy sector were divided and compiled into separate databases to determine the most effective and impactful mitigation strategies. Overall, the use of 3NOP and marine algal strains as dietary additives were identified as the most promising technologies in reducing enteric fermentation, without negatively impacting production markers and subsequent profit. Tanniferous legumes as a forage also shows promise, however current findings in research demonstrate mixed effects on various production markers in dairy and beef cattle. Other mitigation strategies identified through the review process, including the use of various natural and synthetic dietary additives, require further investigation as inconclusive and insignificant results are predominant. To drive adoption of methane reduction strategies by farmers, introduction of the mitigation technologies and strategies discussed must align with Federal and Provincial policy development and implementation and ensure sufficient profit to producers, potentially through the sale of carbon offsets as the market development, in order to cover additional costs of adoption and incentivise use. Prompt introduction and adoption of the mitigation strategies discussed can effectively reduce enteric methane emissions in Canadian cattle herds, propelling Canada towards the 30 percent emission reduction goal in time for 2030.

Keywords: Methane, Enteric fermentation, 3NOP, Asparagopsis, Cattle production, GHG, Global Methane Pledge

INTRODUCTION

Increasing environmental, social, and political pressures around global warming and climate change are driving carbon emissions reduction technologies (Ogunbode et al., 2020; Rauw et al., 2020)). In late 2021 at the COP26 climate conference Canada committed to a 30 percent reduction of methane emissions by 2030 in an effort to mitigate greenhouse gas (GHG) emissions (Environment and Climate Change Canada, 2021; "Much of World Signs up to Global Methane Pledge," 2021). By economic sector, agriculture is responsible for 10 percent of Canada's total emissions, emitting 69 Mt CO_2 eq (Environment and Climate Change Canada, 2022). From the most recent International Panel on Climate Change (IPCC) report demonstrated in Table 1, methane emissions make up over 70 percent of the total emissions from the agricultural sector, virtually entirely derived from livestock operations and production. Over 85 percent of emissions from livestock are derived from enteric fermentation, almost exclusively from cattle.

Cattle are subdivided into *dairy* and *non-dairy* cattle in IPCC National Inventory Reports (NIR) Common Reporting Framework (CRF) tables demonstrated in Table 2, based on production purposes and output. On average, dairy cattle produce 142.93 kg CH_4/head/year, and non-dairy cattle produce 71.05 kg CH_4/head/year. However, since the current non-dairy herd population is more than ten-times larger than the dairy herd, and subsequently is linked to almost six-times more CH_4 emissions per year, displayed in Table 2.

Methane is an especially potent GHG relative to CO_2 as uptake or offset is not possible through photosynthesis but has a shorter atmospheric half-life (Badr et al., 1991). This makes methane emissions derived from the agricultural sector an especially relevant target for reduction, as abatement would have potential major impact on climate change in the short-term. The sector is uniquely posed to reduce greenhouse gas emissions, specifically methane as a result of cattle enteric fermentation, through changes in cattle diet and operation management strategies (Black et al., 2021; Caro et al., 2016).

Cattle, as ruminants, naturally eructate methane as a product of normal feed digestion. Methane production can depend on multiple factors, including dietary components (i.e., forage: concentrate ratio, forage type, fiber content), cattle breed, and management (Alemu et al., 2017; de Faria Maciel et al., 2019; van Gastelen et al., 2019). Each of these factors present distinct areas for novel methodologies and techniques in reducing methane production depending on current individual farm infrastructure and practices.

Reducing methane emissions from dairy and beef cattle production in Canada, especially western Canada, is a focal point of current and future initiatives and subsidies. In Alberta, provincial initiatives like the Quantification Protocol for Selection for Low Residual Feed Intake (L-RFI) in Beef Cattle target reduction of direct and indirect greenhouse gas emissions through cattle breeding and manure management (Alberta. Alberta Environment.,2012). In the pipeline, several novel technologies are being researched and developed globally for preliminary implementation and eventual widespread application (Black et al., 2021).

The measures for agricultural GHG emissions and emissions derived from the enteric fermentation of cattle should be considered with caution. Although approaches to measure enteric fermentation are included in the International Panel on Climate Change 2019 Refinement methodology, aggregated national emission reports have variable margins of error (Dong et al., 2006). The different Tier 1, 2 and 3 methodologies potentially obfuscate true emission values and comparability between emission reports at regional and national levels (Hristov et al., 2018).

This report reviews current literature to identify possible mitigation and adaptation methodologies and technologies across selected dimensions to determine applicability and efficacy on reduction of enteric methane in dairy and beef cattle operations. Among the selected dimensions, factors associated with dairy and beef production operations in alignment with practices in western Canada and specifically Alberta, were selected for throughout the literature review.

METHODOLOGY

In collecting literature and review, a PRISMA approach was employed to assess the relevancy of included papers through the reviewing process. Studies from North America, South America, Europe, Asia, and Oceania were included to explore differing technology in various international operations and production settings. Although different mitigation strategies were considered across multiple dimensions, ultimately the aim of the reviewers was to determine feasibility and application in western Canada and Alberta, despite the influence of climactic conditions and variability like temperature and air moisture content on cattle operations and emissions (Bell et al., 2012; Cullen et al., 2016; Mazzetto et al., 2014).

In the screening and full text review stages, the categories listed below were developed to filter literature and provided the basis for data base collection and extraction. The inclusion and exclusion criteria defined within the category, indicated in italics, was used to facilitate keyword recognition and identification of relevant articles.

After screening using the key word criteria, reviews screened articles based on the dimensions listed below. All articles were screened by a total of three reviewers at the title and abstract stage, and by two reviewers at the full text stage. Screening was completed through blind voting by each reviewer independently in Covidence (Covidence systematic review software, Veritas Health Innovation). The basis of the inclusion and exclusion criteria, in addition to title, abstract and full text screening, was to ensure included articles were relevant to Alberta and western Canada production and operations.

Overall, Covidence imported 1907 references for screening, removing 428 duplicates. The title and abstract were screened for 1477 studies, and 1168 studies were excluded. This left 309 studies for full text screening, of which 109 were considered unfit for extraction. The final database compiled for extraction totaled 189 articles: 84 related to dairy production and operations, and 56 related to beef production and operations. The remaining 49 review and meta-analysis literature was kept as reference material for this report.

SPECIES AND CATTLE BREED

As cattle production is responsible for almost the entirety of methane emissions through enteric fermentation, the research population was limited to cattle, and did not include other ruminant species, such as sheep, goats, and bison. Breeds were selected to reflect current Canadian breed types in dairy and beef production, as breed can influence methane production characteristics in cattle breeds (*Canadian Beef Breeds Council*,2022; *Holstein Canada: About Us - The Canadian Dairy Industry*, 2015.; Islam et al., 2021; Olijhoek et al., 2018; van Gastelen et al., 2019). *In vivo* trials were included, and *in vitro* trials were screened out, to help identify applicable methodologies further in development.

Breeding methods involving the selection of traits in cattle to change enteric fermentation and methane emissions in offspring were not included in this review. Although selection for traits in cattle to reduce enteric methane emissions in progeny has demonstrated success and is part of a provincial mitigation strategy in Alberta, the review aims to collect research interventions that are more accessible on farm to producers and cattle owners and will yield reduction results in the short-term horizon (Alberta. Alberta Environment., 2012.-b; González-Recio et al., 2020).

Inclusion screening tags: *Holstein, Ayrshire, Jersey, Angus, Hereford, Simmental, Charolais, Limousin, short horn, Gelbvien, vivo, Canada, Australia, United States.*

Exclusion screening tags: *Zebo, Hawoo, Wagyu, Brahman, Bos indicus, vitro, sheep, goat, bison, genetics, sequencing, lab*

FEED INGREDIENTS

Environmental and climactic conditions in Alberta and western Canada impact the capacity to grow certain forages and fodder appropriate for animal feed, based on the 34 plant types in Alberta (Shen et al., 2019). The use of certain feed ingredients uncommon or not readily available in Alberta and western Canada were filtered out when included as a main ration component (e.g., tropical forages, coconut meal, citrus pulp or derivatives, almond hulls). Addition of additives or changes in feed regime were screened to only include trials that were administered through feed and did not include trails involving administration though direct ruminal cannula administration or dosage, with the exception of one article that utilised 3NOP administration.

HOUSING AND PASTURE

Housing and access to pasture in feeding trials was an especially prohibitive criteria in dairy cattle production. Canadian dairy parlours commonly use tie-stall housing for lactating heifers and cows across the nation. Studies involving dairy cattle in grazing or pasture-based operations were not included for paper extraction. Studies with beef cattle included grazing steers and confined high-grain rations in feedlots.

Based on these criteria and system review, journals were collected and compiled. Data was extracted from each article to build a data base to support analysis and provide an understanding of current research in the dairy and beef sector, where and how this research was conducted, methane measurement technologies and duration, and methane reduction measurements. This extracted data formed the basis of the review and analysis presented in this report.

RESULTS AND DISCUSSION

The goal of this review was to analyze published data related to mitigation of enteric methane (CH_4) emissions from dairy and beef cattle and document the most effective and sustainable strategies of enteric CH_4 reduction. The database for this research was made up of 189 studies and reviews compiling significant results of research on enteric CH_4 mitigation strategies from many regions of the world, with a focus on North America. With this in mind, the right mitigation approach aims to adapt to the specific needs of the farmers and animals (Knapp et al., 2014). Most importantly, if farmers are to be convinced to adopt these strategies, mitigation tactics will need to be cost effective or cost neutral.

The mitigation strategies examined in this report included (i) 3-nitrooxypropanol (3NOP), (ii) seaweeds and algae, (iii) tannins, iv) forage, grains and other components, v) dietary lipids, vi) nitrate, (vii) essential oils, (viii) natural and synthetic additives, ix) yeast.

The studies surrounding each mitigation strategy considered the geographic location, breed of cow, sample size, trial length, method of enteric CH_4 measurement, and duration of measurement. The average sample size of beef cattle was 33 with experimental herds ranging from 4 to 326. Similarly, the average size in dairy cattle was 26 ranging from 4 to 365. Trial periods also varied, ranging from 14 days to 3 years in length. Enteric CH_4 for each experiment was collected using either the sulfur hexafluoride tracer gas (SF6) technique, the Greenfeed Emission Monitoring system, respiratory chambers, or a combination. All devices are considered acceptable methods of reporting enteric CH_4 emissions, despite potential variances between methods (Jonker et al., 2016).

From the 140 studies considered, incorporating 3NOP and seaweeds into cattle diets showed the greatest and most consistent results of reducing enteric CH_4. Others show promise as enteric CH_4 mitigation agents, however further research will be required in the future to provide conclusive results.

3-NITROOXYPROPANOL (3NOP) AS A FEED SUPPLEMENT

The investigational product 3NOP is an enteric CH_4 inhibitor developed by DSM Nutrition Products Ltd (DSM, 2019). This feed additive is highly soluble and rapidly metabolised in the rumen where it has its beneficial effect. It has been shown to be an effective enteric CH_4 mitigant with consistent effects across studies regardless of animal species and diet composition (Dijkstra et al., 2018). The lowest proposed commercial dose of 3NOP (60 mg/kg DM of the total daily ration) when applied to TMR can reduce enteric CH_4 emissions from dairy cows by 22–35 percent (DSM, 2019).

An evaluation of 14 (7 dairy, 7 beef cattle) of the 140 total screened studies in the database used 3NOP as the enteric CH_4 mitigation technique. All the studies showed that a supplementation of 3NOP was significant in reducing methane emissions in cattle regardless of breed, production, and length of experimental period. 3NOP treatment did not have an apparent effect on milk yield, body weight change, or body condition score, though select studies highlighted that further research is needed to determine the effects of 3NOP dose on weight gain, feed conversion efficiency, and carcass characteristics to further support future widespread adoption of this enteric CH_4 reduction practice. Currently, 3NOP is approved for use in Brazil and Chile, and was reviewed by the European Food Safety Association for use in the European Union in late 2021 and received market approval in February 2022 (Bampidis et al., 2021; Heerlen, 2022).

Various methods of providing 3NOP to ruminant livestock have been used (see Table 3): 3NOP mixed into a TMR, pumped directly into the rumen at feeding time through rumen cannula, top dressed onto feed, incorporated into a concentrate pellet, and added to the roughage component. 3NOP was shown to be effective in all of these methods but mixing to the total mixed ration (TMR) or a component of the ration was most consistent and applicable in practice. Mixing in TMR may allow for continual uptake of the inhibitor throughout the day. Several studies demonstrated once 3NOP is removed from the diet, its effect on enteric CH_4 is negated over time. Melgar et al., (2021) found that the maximum mitigation effect of 3NOP (45 percent decrease) was observed immediately after feeding, persisted 10 h after feeding (an average of 35 percent decrease), decreased beyond 20 h after feeding (average 13 percent decrease), and was nonexistent 2 h before next feeding, which is consistent with the concept that 3NOP must continuously enter the rumen to be an efficient mitigant (Melgar et al., 2021).

Inclusion of 3NOP in ruminant diets was found to decreases enteric CH_4 emissions in a dose–dependent response manner. From the studies in the collected database, the average 3NOP dose used in cattle was 98 mg/kg of DM, ranging from 40 to 200 mg/kg of DM. The average dosage was higher in beef cattle than dairy, reporting 141 mg/kg of DM, ranging from 100 to 200 mg/kg of DM. The average dose in dairy cattle was 56 mg/kg DM. Several studies report that increasing dosage level of 3NOP linearly decreased enteric CH_4 emissions. The study by Hristov et al., (2015) used two different enteric CH_4 measurement techniques (Greenfeed and Sulfur Hexafluoride Tracer (SF6) technique) with doses of 40, 60, and 80 mg/kg DM. Compared with the control, 3NOP decreased the average CH_4 emission by 25 percent, 31 percent, and 32 percent respectively using the Greenfeed method. A similar decrease in enteric CH_4 emission by 3NOP was also observed using the SF6 technique. Compared with the control, 3NOP decreased average enteric CH_4 emission by 20 percent, 25 percent, and 29 percent, respectively. Vyas et al. (2016) also reported a linear effect of 3NOP dose between 100 and 200 mg/kg DM on CH_4 yield (g/kg DMI, maximum decrease of 45 percent) in feedlot cattle. In contrast, no linear response to 3NOP concentration was observed in beef cattle by Alemu et al. (2021). One of four treatments ranging from no to high (150mg/kg DM) levels of 3NOP were randomly fed to a study of 100 crossbred steers. Compared with the control (10.78 g/kg DMI), CH_4 yield (g/kg DMI) was decreased by 52 percent, 76 percent, and 63 percent for low, medium, and high doses of 3NOP, respectively. Reasons for this are not clear.

When examined across the selected studies, the efficacy of 3NOP in decreasing CH_4 emissions was similar between dairy and beef cattle. Conversely, Kim et al., (2020) stated that the effects of CH_4 mitigation by increasing levels of 3NOP supplementation in dairy cattle were more critical than those in beef cattle. This meta-analysis indicated that the appropriate level of 3NOP to reduce enteric CH_4 emissions may vary depending upon the animal type. The same result was not found in the current selection of studies. Kim et al., (2020) predicted dosing 100 mg 3NOP/kg DMI would decrease enteric CH_4 emissions in dairy cattle by 36.4 percent compared with 17.3 percent in beef cattle. According to equations in the meta-analysis, a dose of 60 to 80 mg 3NOP/kg DMI for dairy cows and 150 to 200 mg 3NOP/kg DMI for beef cattle would be expected to decrease enteric CH_4 emissions by 30 percent.

As previously reported, all of the studies evaluated showed a decrease in enteric CH_4 production when 3NOP was supplemented, however the scope of CH_4 emission reduction ranged from 18 percent (Seon-Ho et al., 2019) - 76 percent (Alemu et al., 2020), with the average reduction of 33 percent. Factors causing variability in the response to 3NOP among the studies may be related to feed type. Based on the review by Yu et al., (2021) in the same cattle type, the mitigation effect of 3NOP has been greater in high concentrate diets and less in high fiber diets. For example, several studies using 3NOP as a feed additive have reported very high reductions in CH_4 emissions from feedlot cattle fed grain-based diets (76 percent in Alemu et al., 2020 fed a corn-based diet and 59.6 percent in Romero-Perez et al., 2015 when fed a TMR with 35 percent barley grain.)

Other factors may be related to the variability in results are: method used to measure CH_4 emissions (chambers, Greenfeed system, and SF6), duration that cattle were fed 3NOP, and interaction effects when 3NOP was combined with other mitigation strategies or products. For example, in the study by Zhang et al., (2021) 50g/kg of canola oil was mixed into the TMR with 200mg/kg of 3NOP. The mixture resulted in a higher percent decrease (51 percent) in CH_4 emissions when compared to exclusively canola oil (27.4 percent) and 3NOP (31.6 percent), potentially indicating an additive effect.

Although all the research found demonstrates positive results, 3NOP is not yet available in North America and will not be for some time. DSM's feed additive, given the brand name Bovaer, received market approval in February 2022 in the EU; the first time a feed additive was authorised in the EU for environmental benefits (Heerlen, 2022). This milestone will hopefully function as a model for Canadian regulatory approval in the near future.

SEAWEED AND SEAWEED BIO-ACTIVES AS A FEED SUPPLEMENT

Seaweeds are diverse plants containing bio-actives that are increasingly under investigation as a feed supplement for the mitigation of enteric CH_4 (Abbott et al., 2020). The limited data available indicate dietary supplementation with seaweed produced a significant and substantial reduction in CH_4 yield. An evaluation of 2 studies (Kinley et al., 2020; Roque et al., 2019) and 3 meta-analyses concluded that while there is evidence of benefit from seaweed use to reduce CH_4 yield, further research and trials are required to strengthen the evidence that benefits would also be observed in Canada.

More than 21 seaweeds have been shown to reduce enteric CH_4 emissions. Red (*Asparagopsis taxiformis* (*A. taxiformis*)) and brown (*Ascophyllum nodosum*) seaweeds prove to be the dominant choices, while others have no reported mitigation effect at all. Incorporated into a high grain TMR, *A. taxiformis* was fed to Australian Brahman-Angus cross steers at 0.00 percent, 0.05 percent, 0.10 percent, and 0.20 percent of feed organic matter. Steers receiving 0.10 percent and 0.20 percent of *A. taxiformis* demonstrated decreased enteric CH_4 emissions up to 40 percent and 98 percent, and weight gain improvements of 53 percent and 42 percent, respectively. There was no negative effect on daily feed intake, feed conversion efficiencies, or rumen function, and no residues or changes in meat quality were detected (Kinley et al., 2020).

Similarly in California, a decline in enteric CH_4 of 67.2 percent and 26.4 percent was observed when a very closely related species to *A. taxiformis*, *Asparagopsis armata* (*A. Armata*), a red algae, was fed to Holstein dairy cattle at inclusion levels of 1 percent and 0.5 percent, respectively (Roque et al., 2019). No significant body weight change or milk yield difference between cows receiving *A. armata* at low inclusion compared to control; however, cattle receiving the 1 percent level gained 9.72 kg less than control cattle and produced 11.6 percent less milk.

McCaluey et al., (2020) found dosage amount to be crucial, with higher concentrations of algae in the diet tending to decrease DMI, milk production and substrate digestibility. Dosage of algal feed greater than 15 percent in the diet (DM basis) also tended to affect the palatability and overall intake. Dairy cows offered diet supplements with 6.5 percent or more of *A. armata* regularly refused feed and selected against these feeds (McCaluey et al., 2020). The trade-off between the supplemented dosage to support maximum CH_4 reduction versus the amount tolerated by cattle must be balanced carefully (McCauley et al., 2020).

The potential of seaweed as a feed additive to reduce enteric CH_4 emissions from ruminants depends on a number of factors including the level of the bioactive compound present in the seaweed, which in turn is dependent on seaweed availability and sustainability, harvesting, transport, storage, and processing methods employed to formulate seaweed into a feed ingredient (Abbott et al., 2020). *Asparagopsis* (a genus of red macroalgae) used in experiments is typically grown in warmer climates and has been collected manually in the wild. This process is expensive and not practical for commercial application (Black et al., 2021). Due to its small size, harvesting and drying is difficult and labour intensive. This creates a large environmental footprint (McCauley et al., 2020). Commercial production of *Asparagopsis* in algae ponds in Tasmania has begun due to the enormous potential to virtually eliminate CH_4 emissions from cattle (McCauley et al., 2020). There are also several new projects in New Zealand and in the USA, targeted at understanding the history of this algae and how to commercialize it (McCauley et al., 2020). Although there is ongoing research to incorporate Canadian grown seaweed into cattle diets on Canada's East and West coasts, farmers on the prairies will likely be resistant about using seaweed in lieu of traditional feeds that grow in the immediate vicinity due to the additional costs for transport and of processing seaweed species.

TANNINS AS A FEED SUPPLEMENT

Dietary tannins' supplementation has received special attention, particularly in ruminants. Tannins are a group of polyphenolic compounds that are present in a wide variety of plants and can have positive effects on digestive health, the immune system and provide antiparasitic and anti-inflammatory effects for animals (Orzuna-Orzuna et al., 2021). Tannins are generally classified based on their chemical structure into two groups: condensed tannins' (CTs) and hydrolysable tannins' (HTs). The meta-analysis by Orzuna-Orzuna et al., (2021) showed that tannins' have been successfully used to reduce enteric CH_4 production. Several studies demonstrate an inverse relationship between tannin dietary supplementation and enteric CH_4 emissions per day and per unit of dry matter intake.

The studies included in the database were conducted in 4 different countries: Canada (3), United States (3), France (1), and Switzerland (1). The experimental doses of tannins ranged from freely grazed, offered as hay, or a small percentage (DM) of the diet (0.25 percent-1.5 percent). The tannins used were divided into CTs, HTs, or a mixture of both. Of the included studies, 3 used CTs, 2 used HTs, and 3 used mixtures of CTs and HTs, see Table 4. Conversely, there was a 50-50 split between using tannins extract in the diet and using parts of plants, forages, or subproducts that contained tannins in natural form. The tannins' sources came from a wide variety of trees including chestnut, quebracho, birdsfoot trefoil, sainfoin, small burnet, oak, and hazel plant. Enteric CH_4 was measured using either respiration chambers, Greenfeed system, or SF6 technique and the duration of CH_4 measurement ranged from 2 to 21 days.

The results from the studies ranged from very minimal effects on CH4 production to significant effects. The discrepancies between the studies may be due to the differences in the variability of tannin type, structure, and source.

Chung et al., (2013) found that CT-containing sainfoin had minimal impact on enteric CH_4 emissions from beef heifers when compared with alfalfa (or 80 percent alfalfa, 20 percent sainfoin, as-fed basis for fresh forages). Ebert et al., (2017) found a lack of incremental CH_4 mitigation by CT when fed three different supplements (0 percent, 0.5 percent, 1 percent DM of diet) of quebracho tannin extract. It was suspected that this may be due to the compounds in the basal diet: steam-flaked corn grain, high-fat corn grain by-products, fat, and an ionophore, which are alleged to already decrease methanogenesis. It is important to note that its effect on CH_4 production has been inconsistent and appears to be transitory in nature (Ebert et al., 2017).

In contrast, Aboagye et al., (2018) reported that although daily CH_4 production was not affected by tannin supplementation, CH_4 yield (g/kg DMI) tended to decrease by 6 percent with supplementation of 1.5 percent chestnut + quebracho tree relative to the control. Terranova et al., (2021) also found that CH_4 emissions clearly and substantially declined when dried alfalfa was replaced with tannin-rich leaves of hazel, supporting the hypothesis that increasing the proportion of hazel leaves in the diet would decrease enteric CH_4 yield. Stewart et al., (2019) also found that CH_4 emissions from cattle was lower for small burnet (a tannin-containing hay) than alfalfa (a non-tannin-containing hay).

Under the conditions of the experiments, none of the studies reported to have negative effects on growth performance, nitrogen balance, or energy partitioning of cattle. In fact, Lagrange et al., (2020) reported heifers grazing tanniferous legumes (1.05kg/d) had average daily gains 40 percent greater than heifers grazing alfalfa (0.74kg/d) during the first year. Conversely, a review by Hristov et al., (2013) found that in some situations intake and milk production may be compromised. The majority of studies on plant tannins have measured CT concentrations in plants grown in a semi-arid or tropical region, but only limited data has been collected on CT concentration of forage legumes grown in a temperate climate, such as in western Canada (Berard et al., 2011). Plant tannin concentrations from semi-arid or tropical regions are considerably higher than those observed in western Canada so it is important to note that the studies examining CT concentrations in other countries may not reflect growth conditions in Canada (Berard et al., 2011).

FEEDING FORAGES, GRAINS, AND OTHER COMPONENTS

Although reductions in CH_4 emissions are less drastic than supplementation of 3NOP or seaweed, increasing forage digestibility and digestible forage intake was one of the major recommended CH_4 mitigation practices in a review by Hristov et al., (2013). While responses vary, CH_4 emissions can be reduced when corn silage replaces grass silage in the diet. Hammond et al., (2016) found in two experiments (using either GreenFeed or respiration chambers for CH_4 emission measurements) that compared with high amounts of grass silage, dairy cows fed high amounts of corn silage had a higher DMI, greater milk production, and lower CH_4 yield (24 percent lower in Experiment 1 using Greenfeed and 8 percent lower in Experiment 2 using respiration chambers.). Similarly in a Canadian study, Benchaar (2014) found that mitigation of enteric CH_4 production could be achieved by increasing the corn silage proportion at the expense of barley silage in dairy cattle diets. However, an evident decline (-14 percent) in enteric CH_4 energy loss was observed only when corn silage entirely replaced barley silage in the diet. O'Neil (2012) found that a partial mixed ration (containing 450g of corn silage) actually increased CH_4 production compared with various grass-based diets but this is solely attributed to the increased DMI rather than to any particular nutritional characteristic of the partial mixed ration.

Feeding leguminous forages could also lower CH_4 emissions compared to oat hay due to lower fibre concentration. Replacing oat hay with different proportions of either alfalfa hay or common vetch hay is shown to be beneficial in reducing CH_4 emission in cross-bred Simmental cattle. Du et al., (2019) 's findings suggested that substituting 20 percent alfalfa hay and 40 percent common vetch hay for oat hay are the optimal proportions to maintain body weight gain and reduce CH_4 emissions.

The effects of feeding wheat on enteric CH_4 reduction have also been explored. Moate et al (2017) fed Holstein cows one of 4 diets: corn, wheat, single rolled barley, and double rolled barley. The SF6 tracer technique was used to estimate CH_4 emissions for all cows. The results showed that the mean CH_4 emissions and CH_4 yields of cows fed the wheat diet were significantly less than those of cows fed the other diets. Corn, single rolled barley, and double rolled barley diets were associated with 49 percent, 73 percent, and 78 percent greater CH_4 emissions, respectively, compared with the emissions from the wheat diet (Moate et al., 2017). It is important to note that although this study showed including wheat in the diet of dairy cows could be an effective strategy for reducing CH_4 emissions, it also reduced milk fat percentage and production of milk fat and energy-corrected milk (Moate et al., 2017).

The compiled database Table 4 contains numerous references on effects of forage quality, pasture management, and processing CH_4 in beef and dairy cattle. In Canada, cattle are commonly fed grasses (natural grasses, alfalfa, corn stalk) in the form or dry hay and silage. They also can get their nutrients from crops such as corn, barley, alfalfa hay, oats, and soybeans (DFC, 2019). In general, CH_4 reductions are correlated with greater nutrient quality and digestibility, the impact on CH_4 mitigation, when scaled per unit of animal product, should be typically greater when cattle consume higher quality forage (Hristov et al., 2013).

FEEDING DIETARY LIPIDS

Dietary lipids can be effective in reducing CH_4 emissions, but applicability depends on feed intake, fiber digestibility, production, and target milk composition. Winders et al., (2018) fed corn oil at 3 percent of a mostly corn based diet DM to beef steers and found it reduced enteric CH_4 production by 14.6 percent, but this was partially due to a 4.4 percent decrease in DMI. Alvarez-Hess et al., (2019) found that increasing the fat concentration from 2 to 6 percent DM reduced CH_4 per kg of DM intake regardless of the grain component in a dairy cow basal diet. While Munoz et al., (2019) evaluated a variety of unprocessed oilseeds on dairy cattle and found that cottonseed oil decreased CH production (g/d) and yield (g/kg of DMI) compared to rapeseed and linseed oil. Although dietary lipids may be beneficial as CH_4 mitigating agents, some fats such as coconut oil for example, can severely depress feed intake, fiber digestibility, and consequently, milk production and cause milk fat depression in dairy cows (Hristov et al., 2013).

Two Canadian based studies looked at supplementing diets with commercially available lipid sources with potential to reduce enteric CH_4 emissions from cattle. Chung et al., (2011) investigated potential effects of feeding ground linseed on enteric CH_4 production when it was added to diets containing grass hay or barley silage. Results showed that including linseed in the hay-based diet did not supress CH_4 emissions, whereas including linseed in the silage-based diet reduced enteric CH_4 emissions by 33 percent CH_4/kg DM intake. It is thought that the differing effects of linseed on diet type may be due to the difference's linseed has on ruminal fermentation and ruminal digestion of forages. Beauchemin et al., (2007) found that both sunflower oil and sunflower seeds had good potential for on-farm adoption as they both reduced CH_4 by 17 percent expressed on the basis of digestible energy intake. However, these ingredients may increase the cost of feeding, thus use of lipid feeding on commercial beef cattle farms for CH_4 reduction will depend on the economic benefits or risks linked to effects on animal performance (Beauchemin et al., 2007). Dietary lipids may be beneficial as CH_4 mitigating agents, though some fats such as coconut and sunflower oil for example, can severely depress feed intake, fiber digestibility, and consequently, milk production and cause milk fat depression in dairy cows (Hristov et al., 2013). Further research is required to investigate ways to simultaneously mitigate CH_4 emissions and maintain or improve milk quality and output.

NITRATE AS A FEED SUPPLEMENT

Lee et al., (2017) found that supplementing encapsulated nitrate (EN) as a salt to beef cattle did not result in clear statistical differences in CH_4 production among treatments. Enteric CH_4 production decreased up to 17 percent and CH_4 yield reduced by 6 percent when fed at 2.5 percent EN compared to the control, but these decreases were not statistically significant. In contrast, Lund et al., (2014) found that average CH_4 production per day decreased by 31 percent as a result of incorporating nitrate in the diet and subsequently increased by 34 percent when nitrate feeding stopped. It is important to note that introducing nitrate to cattle reduced feed intake in the first hours after introduction, but when corrected for the decrease in DM intake, CH_4 production was significantly reduced by 25 percent.

Nitrates show promise as CH_4 mitigation agents, however, decreases in DMI resulting from a combination of factors have often been observed in multiple studies. It is hypothesized that when nitrate is added to the diet, a change in organoleptic properties is the major reason for lowered feed intake in ruminants, but the answer is not definitive (Lee et al., 2017). Nitrates are not commercialized yet in Canada mainly because of the potential of nitrate toxicity (Lee et al., 2017). This significant drawback has limited the use of nitrate as a CH_4 mitigation strategy. More research and studies are needed to fully understand their impact on whole-farm greenhouse gas emissions, animal productivity, and animal health.

ESSENTIAL OILS AS FEED SUPPLEMENTS

A meta-analysis by Belanche et al., (2020) discusses several studies demonstrating the potential of essential oils (EO) on decreasing CH_4 production in vitro, however few studies have evaluated the effects of EO and their constituents on CH_4 emissions in vivo. The meta-analysis highlights the challenging of finding a combination of EO that reduces CH_4 production without also decreasing feed intake and productivity.

A variety of EOs were used in the studies collected from the database, including oregano oil, cinnamon oil, and Agolin – a commercial essential oil feed additive. The meta-analysis by Belanche et al., (2020) identified 23 studies that supplemented Agolin (AGO) at 1g/d per cow and found that short term studies showed minor and inconsistent effects of AGO whereas long term studies (an adaption period of at least four weeks of treatment) of AGO supplementation decreased CH_4 production per day (-8.8 percent). AGO was also found to increase milk yield (+3.6 percent) and feed efficiency (+4.4 percent) without further changes in milk composition and feed intake. In contrast, a study by Carrazco et al., (2020) found that CH_4 production was found to be similar between AGO-treatment and control cattle. The discrepancy in the studies may be linked to the study design, as treatment was applied to individual animals and the treatment and control animals were not separated. Researchers therefore could not ensure that each animal consumed the allocated 1 g/head/day of AGO in this model (Carrazco et al., 2020).

Benchaar (2020) and Benchaar (2016) both found no effect on CH_4 production when supplementing oregano oil and its main component carvacrol, and cinnamon oil, respectively. When supplemented at 50mg/kg of DM, oregano oil and carvacrol are likely not a viable strategy to improve feed efficiency, mitigate enteric CH_4, or enhance milk performance in dairy cows (Benchaar 2020). Subsequently, feeding 50mg/kg DMI of cinnamon oil was not an effective strategy to reduce CH_4 emissions in dairy cows.

Several studies have showed that EO can decrease CH_4 production in vitro in a dose dependent manner (Belanche et al., 2020), however evidence for in vivo studies of essential oils has been ambiguous to date, this may be due to the capacity of rumen microbes to adapt and degrade these substances (Benchaar, Greathead, 2011). Further, many of the concentrations of EO's that have favourably affected rumen fermentation in vitro are too high for in vivo use and would likely have negative effects on efficiency of rumen fermentation, palatability and possibly cause toxicity (Benchaar, Greathead, 2011). The challenge consists in finding a combination of EO's that reduce CH_4 production with lasting effects without an associated decrease in feed intake and cattle productivity. Based on available results, there is a need for in vivo investigation to determine whether these EO's can be used successfully to inhibit rumen methanogenesis.

SYNTHETIC AND NATURAL ADDITIVES AS FEED SUPPLEMENTS

A meta-analysis by Appuhamy et al., (2013) studied the effects of the feed additive monensin in dairy cow and beef steer diets. The monensin effects in dairy cows were notably inconsistent, as an almost equal number of studies had positive and negative monensin effects on CH_4 production. Monensin had a more consistent effect on CH_4 mitigation in beef steers, but the effect sizes remained variable across studies. Beef steer diets were supplemented with higher monensin doses (average = 32 mg/kg of DM) for relatively short periods of time (average 34 days) compared with low monensin doses in dairy cows (average = 21 mg/kg of DM) fed for a longer period of time (average 85 days) (Appuhamy et al., 2013). Similarly, Grainger et al., (2010) fed dairy cows 20 to 23 mg/kg of DMI and concluded that no short- or long-term reductions in enteric CH_4 emissions were noted per day, per kilogram of DMI, or per kilogram of milk. Stackhouse-Lawson et al., (2013) fed a treatment combination of monensin and tylosin phosphate and found when emissions were calculated based on gram per kilogram hot carcass weight per day, treatment steers had lower CH_4 emissions per kilogram compared to steers fed the control diet of steam flaked corn and alfalfa hay.

Other additives (natural and synthetic) were assessed to reduce CH_4 emissions and had varying success rates. Two natural additives, chitosan (Jimenez-Ocampo et al., 2019) and humic substances (Terry et al., 2018) both reported no effect on enteric CH_4 production in cattle when fed at inclusion levels of 0.5 and 1.0 percent of the diet and levels 100-, 200-, or 300-mg, respectively. In contrast, 40mg Copper (Cu) (from supplemental $CuSO_4$)/kg supplemented to Holstein bulls decreased CH_4 emissions by 23 percent compared to the control (20mg Cu) diet. However, information on the potential of micromineral supplements in this regard is limited (Sanchez-Sanchez et al., 2021).

YEAST AS A FEED SUPPLEMENT

Some direct-fed microbials, such as yeast-based products, might have a moderate CH_4-mitigating effect through increasing animal productivity and feed efficiency, but the effect is likely to be inconsistent, and convincing animal data is lacking (Hristov et al., 2013). The two studies in the database (Li et al., 2021, and Meller et al., 2019) support this theory with both reporting insignificant evidence of live yeast culture and active dry yeast affecting CH_4 reduction on both Jersey and Holstein cows, respectively.

The meta-analysis by Hristov et al, (2013) reported an overall positive of effect of various yeast-based products on milk yield in dairy cows, with one review (Robinson and Erasmus, 2009) increasing milk yield by 3.6 percent on average over the control. The same yeast-based product had no effect on feed intake or milk production and composition of dairy cows (Hristov et al, 2010b) which only emphasizes the variability and conditional effects of these products (Hristov et al, 2013).

CONCLUSION

Reduction of emissions from cattle production in western Canada and Alberta will become increasingly important as environmental, societal, and political pressures rise. Mitigation strategies, depending on factors related to production practices and operation management, may be adopted in the future, however it is clear that there is no “one size” solution across the beef and dairy sectors. The most promising strategies identified through systemic review involve natural or synthetically derived dietary additives, such as inclusion of marine algae or 3NOP in rations or increasing consumption of tanniferous forages and legumes in grazing or rations. Although other technologies and strategies are also being researched, variable results and insignificant effects across studies highlight the need for further investigation into the future.

Next steps of this research would involve development of a feasibility and adoption matrix, to gauge willingness to adopt practices by cattle farmers and producers based on cost, operation, and infrastructure. Additionally, policy measures at the federal level to support registration of feed additives that reduce enteric methane as regulated feed additive in Canada, and accessibility of those additives by farmers should be prioritized. Currently, 3NOP is classified as a veterinary drug and therefore is not approved for use in livestock diet and rations; a critical regulatory barrier that may take years to rectify before the additive can be applied in Canada to mitigate enteric methane production (*Feed Additive Remains Years Away in Canada | The Western Producer*, Arnason, 2022.; *RG-1 Regulatory Guidance:Feed Registration Procedures and Labelling Standards - Canadian Food Inspection Agency*,2022.).

Supporting adoption and implementation by producers in the Canadian beef and cattle sector can result in various future scenarios and impacts on methane mitigation. Both 3NOP and marine macroalgae feed additives have demonstrated enteric reduction potential between 30 to 90 percent (Black et al., 2021). With prompt federal and provincial legislative action, catered to specific production climates and locations, the implementation of these feed additives in the rations of dairy and feedlot cattle can support Canada’s methane reduction target in time for 2030. To drive producers’ adoption and use in on-farm operations, the use of feed additives as an enteric methane mitigation strategy can be combined with the sale of subsequent carbon offsets. This will propel adoption, as increased profits from offset sales can reduce additional upfront feed costs and possible changes in operations.

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TABLES

Table 1: Methane Emissions from Agriculture in Canada

GREENHOUSE GAS SOURCE AND SINK CATEGORIES	CH_4 (kt)
Total Agriculture	1104.34
↳ **Livestock**	1102.71
↳ **Enteric Fermentation**	947.08

Adapted from (Canada, 2022)

Table 2: Methane Emissions from Canadian Dairy and Non-dairy Cattle Herds

GREENHOUSE GAS SOURCE AND SINK CATEGORIES	POPULATION SIZE (1000)	CH_4 (kg CH_4/head/yr)	CH_4 (kt)
Cattle	11750.00	77.01	904.88
↳ Dairy Cattle	973.75	142.93	139.17
↳ Non-dairy Cattle	10776.25	71.05	765.71

Adapted from (Canada, 2022)

Table 3: Compiled 3NOP Database for Beef and Dairy

	Reference	Type/ Stage	Sample Size	CH_4 Measurement Method	Days Measured	3NOP (mg/kg) Administered	CH_4 Application	CH_4 Reduction Percentage
Beef	SeonHo et al., 2019	Beef steers	9	Greenfeed System (C-Lock)	3	100	Pumped Directly into Rumen	18%
	XiuMin et al., 2021	Beef heifers	8	Open Circuit Calorimetry Chambers	4	200	Mixed into TMR	31.60%
	Romero-Perez et al., 2015	Angus heifers	8	Metabolic Chambers	3	129	Mixed into TMR	59.20%
	Vyas et al., 2016	Crossbred steers	15	Calorimetry Chambers	3	100-200	Mixed into TMR	30%
	Vyas et al., 2018	Crossbred steers	20	Calorimetry Chambers	3	200 (backgrounding) 125 (finishing)	Mixed into TMR	42% (backgrounding) 37% (finishing)
	Alemu et al., 2020	Crossbred steers	100	Greenfeed System (C-Lock)	112	Low: 100, Med: 125, High: 150	Incorporated into a Concentrate Pellet	76%
	Dijkstra et al., 2018	Crossbred steers	15	Metabolic Chambers	9	123	Mixed into TMR	N/A
Dairy	Schilde et al., 2021	Holstein	55	Greenfeed System (C-Lock)	92	48.4, 51.2	Mixed into TMR	33%
	Hristov et al., 2015	Holstein	48	Greenfeed System (C-Lock)	3	40,60,80	Top Dressed onto Feed	30%

	Reference	Type/ Stage	Sample Size	CH_4 Measurement Method	Days Measured	3NOP (mg/kg) Administered	CH_4 Application	CH_4 Reduction Percentage
Dairy	Lopes et al., 2016	Holstein	6	Greenfeed System (C-Lock)	3	60	Mixed into TMR	31%
	Melgar et al., 2020	Holstein	56	Greenfeed System (C-Lock)	4	60	Mixed into TMR	26%
	Vanwesemael et al., 2019	Holstein	30	Greenfeed System (C-Lock)	~4	100	Added to Roughage	23%
	Melgar et al., 2021	Holstein	48	Greenfeed System (C-Lock)	~5	60	Mixed into TMR	29%
	Haisan et al., 2014	Holstein	12	Sulfur Hexafluoride Tracer Gas	5	2500 **mg/d**	Top Dressed onto Feed	60%

Table 4: Compiled Tannin Database for Beef and Dairy

	Reference	Country	Type/ Stage	Sample Size	Dose	Measurement Method	Days Measured	Condensed or Hydrolyzable	Tree Type	Extract or Natural
Beef	Aboagye et al., 2019	Canada	Beef Heifers	8	1.43% (of diet)	Respiration Chambers	9	HT	Chestnut	Extract
	Aboagye et al., 2018	Canada	Cross Bred Heifers	15	0.25% - 1.5% DM	SF6	6	Both	Chestnut	Extract (powdered form)
	Ebert et al., 2017	USA	Angus Cross Bred Steer	27	0, 0.5, 1% DM	Greenfeed System	4	CT	Quebracho	Extract
	Lagrange et al., 2020	USA	Angus Heifer	42	Freely grazed	SF6	5	CT	Birdsfoot Trefoil, Sainfoin	Natural
	Stewart et al., 2019	USA	Angus Heifer	9	Fed as hay	SF6	5	Both	Birdsfoot Trefoil, Sainfoin, Small Burnet	Natural
	Chun et al., 2013	Canada	Beef Heifers	8	Offered as hay	Respiration Chambers	8	CT	Sainfoin	Natural
Dairy	Focant et al., 2019	France	Holstein	6	0-414g/kg	SF6	21	HT	Oak	Extract
	Terranova et al., 2021	Switzerland	Brown Swiss and Holstein	20	0-400g/kg DM	Respiration Chambers	2	Both	Hazel	Natural

Table 5: Compiled Alternative Intervention Database for Beef Cattle

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Alemu et al., 2019	Essential Oil	Cross-bred	Steer	88	GreenFeed	112	No
Pinares-Patino et al., 2016	Essential Oil	Cross-bred	Steer	60	SF6	4	Yes
Winders et al, 2018	Lipid	Cross-bred	Yearling Steer	80	Enclosed Methane Barn	15	Yes
Eugenea et al., 2011	Lipid	Charolais	Bulls	56	SF6	15	Yes
Stackhouse-Lawson et al., 2019	Synthetic Additive	Angus Cross	Steer	160	Enclosed Methane Barn	10	Yes
Cooprider et al., 2011	Synthetic Additive	Angus Cross	Heifer	104	Enclosed Methane Barn	5	No
Wang et al., 2015	Synthetic Additive	Simmental	Steer	8	Facemask Technique	6	No
Kinley et al., 2020	Seaweed	Brahman-Angus		20	Respiratory Chambers	4	Yes
Vaizquez-Carrillo et al., 2020	Natural Additive	Charolais x Brown Swiss	Steer	8	Respiratory Chambers	8	Yes
Terry et al., 2018	Natural Additive	Angus x Hereford	Heifer	8	Respiratory Chambers	2	No
Chung et al., 2013	Concentrate		Heifer	8	Respiratory Chambers		No
Lee et al., 2017	Nitrate	Cross-bred	Steer	20	Respiratory Chambers	2	No

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Lee et al., 2017	Nitrate	Cross-bred	Steer	108	Respiratory Chambers		No
Brown et al., 2017	Nitrate	Holstein	Steer	24	SF6	8	Yes
Renand et al., 2019	Forage	Charolais	Heifer	326	GreenFeed		No
Hunerberg et al., 2015	Forage		Heifer	16	Respiratory Chambers	4	No
Jonker et al., 2016	Forage	Hereford x Holstein	Heifer	8	Respiratory Chambers, SF6, and GreenFeed	4	No
Du et al., 2019	Forage	Simmental Cross	Steer	16		3	Yes
Du et al., 2020	Forage	Simmental Cross	Steer	16	Respiratory Chambers		No
Alemu et al., 2017	Forage	Cross-bred	Heifer	16	GreenFeed	72	No
Mahfuzal et al., 2021	Forage	Holstein and Jersey	Steer	12	GreenFeed	3	No
Bouchard et al., 2015	Forage		Yearling Steer	20	SF6	730	No
Chiavegato et al., 2015	Forage	Holstein	Finishing Steer	12	Enclosed Methane Barn	10	No
Boland et al., 2013	Grazing	Limousin Cross	Heifer	30	SF6	10	No
Miku et al., 2022	Grain	Cross-bred	Heifer	16	Respiratory Chambers	4	Yes

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Hunerberg et al., 2013	Grain	Cross-bred	Heifer	16	Respiratory Chambers	4	Yes
Vyas et al., 2014	Grain		Heifer	20	Respiratory Chambers	3	No
Hunerbeg et al., 2014	Grain	Simulated study		9	HOLOS		No
Villar et al., 2020	Grain		Steer	4	Respiratory Chambers	2	No
Min et al., 2021	Grain	Angus Cross	Steer	30	SF6	15	No

Table 6: Compiled Alternative Database for Dairy Cattle

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Benchaar 2016	Essential Oil	Holstein	Multiparous	8	SF6	6	No
Benchaar 2020	Essential Oil	Holstein	Lactating Cow	8	SF6	6	No
Benchaar et al., 2015	Essential Oil	Holstein	Lactating Cow	12	Respiratory Chambers	4	Yes
Darabi et al., 2021	Essential Oil		Cow	4	SF6	6	No
Carraz et al., 2020	Essential Oil	Holstein	Lactating Cow	20	Respiratory Chambers	14	Yes

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Hassanat and Benchaar, 2021	Essential Oil	Holstein	Lactating Cow	12	Respiratory Chambers	5	Yes
Venem et al., 2015	Essential Oil	Holstein and Jersey	Lactating Cow	18	Respiratory Chambers	2	No
Klop et al., 2017	Essential Oil	Holstein	Lactating Cow	8	Respiratory Chambers		No
Alvarez-Hess et al., 2019	Lipid	Holstein	Multiparous	32	SF6	6	Yes
Chung et al., 2011	Lipid	Holstein	Dry Cow	12	SF6	3	Yes
Bougouin et al., 2019	Lipid	Holstein	Lactating Cow	4	Respiratory Chambers	6	No
Munoz et al., 2019	Lipid	Holstein	Lactating Cow	8	SF6	6	No
Moate et al., 2013	Algal Meal	Holstein	Lactating Cow	32	Respiratory Chambers	2	No
Roque et al., 2019	Seaweed	Holstein	Lactating Cow	12	GreenFeed		Yes
Sanchez et al., 2021	Synthetic Additive	Holstein	Bulls	6	SF6	3	Yes
Zijderveld et al., 2011	Synthetic Additive	Holstein	Lactating Cow	12	Respiratory Chambers	7	Yes
Martin et al., 2016	Natural Additive	Holstein	Lactating Cow	8	SF6	4	Yes
Guyader et al., 2016	Natural Additive	Holstein	Lactating Cow	16	Respiratory Chambers	2	Yes

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
XiaoHua et al., 2017	Natural Additive	Holstein	Lactating Cow	10	SF6		Yes
Philippeau et al., 2017	Natural Additive	Holstein	Lactating Cow	8	SF6	6	Yes
Tekip et al., 2011	Natural Additive	Holstein	Lactating Cow	8	SF6	2	No
Sun et al., 2019	Natural Additive	Holstein	Lactating Cow	18	GreenFeed	4	No
Li et al., 2021	Yeast	Holstein	Lactating Cow	60	SF6	4	No
Meller et al., 2019	Yeast	Jersey	Lactating Cow	12	GreenFeed	3	No
Lund et al., 2014	Nitrate	Holstein	Lactating Cow	4	Respiratory Chambers		Yes
Guyader et al., 2015	Nitrate and Oil	Holstein	Non-lactating Cow	4	Respiratory Chambers	4	Yes
Melgar et al., 2020	Nitrate and Oil	Holstein and Holstein x Jersey	Lactating Cow	18	Respiratory Chambers	2	No
Munoz et al., 2018	Concentrate	Holstein	Lactating Cow	24	SF6	7	No
Hassanat et al., 2017	Forage	Holstein	Lactating Cow	16	Respiratory Chambers		No
Lessire and Dufrasne, 2019	Forage		Lactating Cow	6	"Guardian" inserted in feeding bin		No
Coppa et al., 2021	Forage	Holstein	Lactating Cow	45	GreenFeed		No

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Cameron et al., 2018	Forage	Holstein	Lactating Cow	45	Hand-held laser methane detector (LMD)	8	Yes
Bougouin et al., 2018	Forage	Holstein	Lactating Cow	4	Respiratory Chambers	5	Yes
Hatew et al.,2016	Forage	Holstein	Lactating Cow	28	Respiratory Chambers	5	No
Hammond et al., 2015	Forage	Holstein	Heifer	8	Respiratory Chambers	4	No
Benchaar et al., 2021	Forage	Holstein	Lactating Cow	16	Respiratory Chambers	4	Yes
Flay et al., 2019	Forage	Holstein and Jersey	Heifer	56	GreenFeed	25	No
Benchaar and Hassanat, 2021	Forage	Holstein	Lactating Cow	16	Respiratory Chambers	4	No
Mikula el al., 2022	Forage	Holstein and Jersey	Lactating Cow	365	FTIR analyzer	280	Yes
Rico et al., 2016	Forage	Holstein	Lactating Cow	27	Respiratory Chambers	3	No
Huye et al., 2016	Forage	Holstein	Lactating Cow	6	Respiratory Chambers	4	Yes
Holtshausen et al., 2021	Forage	Holstein	Lactating Cow	120	Respiratory Chambers		No
Uddin et al., 2020	Forage	Holstein and Jersey	Lactating Cow	24	GreenFeed		No
Warner et al., 2017	Forage	Holstein	Lactating Cow	56	Respiratory Chambers	5	Yes

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Chung et al., 2011	Forage	Holstein	Non-lactating Cow	12	SF6	2	Yes
Hassanatet al., 2014	Forage	Holstein	Lactating Cow	9	Respiratory Chambers	3	No
Harpe et al., 2017	Forage	Holstein	Lactating Cow	12	GreenFeed	3	No
Hammond et al., 2016	Forage	Holstein	Lactating Cow	44	GreenFeed	2	Yes
Gislo et al., 2020	Forage	Holstein	Lactating Cow	8	Respiratory Chambers	5	No
O'Neil et al., 2011	Forage	Holstein	Lactating Cow	48	Calibrated Tracer Technique	5	Yes
Staerf et al., 2012	Forage	Holstein	Lactating Cow	6	Respiratory Chambers	8	No
Masse et al., 2016	Forage	Holstein	Lactating Cow	9	Wet tip gas meters (manure measurement)	120	Yes
Olijhoek et al., 2018	Forage	Holstein and Jersey	Lactating Cow	20	Respiratory Chambers	3	No
Ellis et al., 2016	Forage	Holstein	Lactating Cow	8	Respiratory Chambers		No
Cueva et al., 2011	Forage	Holstein	Lactating Cow	48	GreenFeed		Yes
Doreau et al., 2014	Forage	Holstein	Lactating Cow	8	SF6	6	No
Chung et al., 2011	Forage	Holstein	Non-lactating Cow	12	SF6	2	Yes

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Cherif et al., 2018	Forage	Holstein	Cow	9	Respiratory Chambers	5	No
Brask et al., 2013	Forage	Holstein	Lactating Cow	6	Respiratory Chambers	4	Yes
Benchaar et al., 2014	Forage	Holstein	Lactating Cow	9	Respiratory Chambers	4	No
Benchaar and Hassanat, 2020	Forage	Holstein	Lactating Cow	12	Respiratory Chamber	5	No
Cameron et al., 2017	Forage	Holstein	Lactating Cow	45	Hand-held laser methane detector (LMD)	8	Yes
O'Neil et al., 2012	Grazing	Holstein	Lactating Cow	48	SF6	5	No
Ferris et al., 2017	Grazing	Holstein	Non-lactating Cow	68	SF6	6	No
Moate et al., 2017	Grain	Holstein	Lactating Cow	32	SF6	5	Yes
Moate et al., 2018	Grain	Holstein	Lactating Cow	24	Respiratory Chambers	2	No
Lopes et al., 2017	Grain	Holstein	Lactating Cow	15	GreenFeed	3	No
Moate et al., 2011	Grain	Holstein	Lactating Cow	16	Respiratory Chambers		No
Niu et al., 2016	Grain	Holstein	Lactating Cow	12	GreenFeed	4	Yes
Benchaar et al., 2013	Grain	Holstein	Lactating Cow	12	Respiratory Chambers	7	Yes

Study Reference	Treatment	Breed	Production Stage	Sample Size	Methane Measurement Method	Days Measured	Significant CH_4 Reduction
Grainger et al., 2010	Grain	Holstein	Lactating Cow	50	Respiratory Chambers	84	No
Borsting et al., 2020	Grain	Holstein	Multiparous	4	Respiratory Chambers	4	No

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