#### GENETIC AND DIETARY STRATEGIES FOR MITIGATING ENTERIC METHANE EMISSIONS IN RUMINANT ANIMAL PRODUCTION: A REVIEW

#### \*Ekanem, N.J., Udoh, J.E. and Afolabi, K.D.

Department of Animal Science, Faculty of Agriculture, University of Uyo, Uyo, Nigeria.

\*Corresponding author's email: <u>ekanem\_n@yahoo.co.uk</u>

## ABSTRACT

Methane (CH<sub>4</sub>) as an important greenhouse gas is detrimental to livestock production and human well-being as it contributes to climate change. The occurrence, sources by sector, mechanism and biochemistry, effects and mitigation strategies of enteric methane emission were hereby reviewed. The Global Warming Potential (GWP) of CH<sub>4</sub> is 28 - 36 times higher than CO<sub>2</sub>. Sectors causing anthropogenic methane emissions include agriculture, energy, industry and waste. Livestock are major sources of methane emissions accounting for between 30 and 50% of the total greenhouse gas emitted. Enteric methane production accounts for about 39 and 80% of the methane emissions from the agricultural and livestock sector respectively. Additionally, methane represents between 2 and 12% loss of gross energy (GE) intake. Strategies for effective mitigation of methane emissions and feed energy loss in ruminant animals include selection and breeding of animals with improved productivity, supplementation with dietary lipids and lipid by-products at optimum level, optimal use of concentrates in the diet, feeding forages with high digestibility and feeding silage instead of hay.

**Keywords:** Greenhouse gases, Global warming, Methane, Enteric fermentation, Mitigation strategies, Ruminant animal

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# INTRODUCTION

Methane (CH<sub>4</sub>) is an important greenhouse gas. Greenhouse gases result in climate changes by affecting the atmosphere chemically in the long term (Dogan, 2005; 2007). Methane is one of the three main Green House Gases (GHG), together with carbon (IV) oxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O). Methane is formed as a result of decomposition of organic materials in an environment without oxygen. It is released from natural and anthropogenic sources. It has been reported that 40% of global methane emissions come from natural sources, whereas 60% of global methane emission is released from anthropogenic sources (USEPA, 2016; Karakurt et al., 2012). Methane as an important greenhouse

gas is detrimental. This is because the Global Warming Potentials (GWP) of N<sub>2</sub>O and CH<sub>4</sub> are 265 and 28 times higher than CO<sub>2</sub>, respectively (IPCC, 2013). USEPA (2016) reported that methane has 28 - 36times global warming potential than carbon dioxide. Also, the life time of methane in the atmosphere is 9-15 years; and over the last methane atmospheric two centuries. concentrations have more than doubled, arising 1 % yearly in comparison with 0.5 % of carbon (IV) oxide (Mirzaei-Aghsaghali et al., 2015). Sectors causing anthropogenic methane emissions include agriculture, energy, industry and waste (EPA, 2006).

Livestock are major sources of methane emission, contributing about 81 to 92 metric tonnes of methane per annum

globally (Patra, 2012; IPCC, 2007). The fore stomach of ruminant animals contain diverse population microbial that produce significant quantities of methane during feed digestion which contributes to greenhouse gas emissions as well as global warming. Enteric methane is the largest source of agricultural emission, accounting for 40 % of total emission (World Bank Report, 2016; FAOSTAT. 2014). Enteric methane accounts for about 80% of the methane emissions from the livestock sector (Karri et al., 2015; Gerber et al. 2013). Methane also represents a significant energy loss to the animal ranging from 2 to 12% of gross energy (GE) intake (Mahesh et al., 2013; Zhi-hua et al., 2012; Johnson and Johnson, 1995). So, decreasing the production of enteric CH<sub>4</sub> from ruminants without altering animal production is desirable both as a strategy to reduce global GHG emissions and as a means of improving animal productivity.

Owing to the loss of feed energy as methane, interest in reducing methane (CH<sub>4</sub>) emissions by cattle has increased recently. Methane (CH<sub>4</sub>) can be mitigated in ruminants through various strategies. Many reviews on the different strategies to CH<sub>4</sub> production mitigate enteric bv ruminants have been published (Karri et al., 2015; Mirzaei-Aghsaghali et al., 2015; Hristov et al., 2013; Buddle et al., 2011; Hook et al., 2010; Beauchemin et al., 2008; Iqbal et al., 2008; Moss et al., 2000). Feed intake and dietary characteristics are the main determinants of methane production and have been widely studied in growing and finishing beef cattle (Johnson and Johnson, 1995). Manipulation of dietary composition has proven to be an effective mitigation strategy. Progress has also been made in identifying nutritional factors that may reduce methane production (Hristov et al., 2013). Thus, this paper will review the contributing different sectors to anthropogenic methane emission and thus contributing to global warming, the

mechanism of enteric methane production and emission, as well as current dietary strategies employed in enteric methane mitigation in ruminant animals.

#### METHANE EMISSIONS FROM VARIOUS SECTORS

The different sectors causing anthropogenic methane emissions include agriculture, energy, industry and waste. Methane emission from energy sector include those from coal mining activities, natural gas and oil systems, stationary and combustion and biomass mobile combustion: while methane emission from industry include those from chemical production, iron and steel production, metal production, mineral products, petrochemical production and silicon carbide production (Karakurt et al., 2012; Gerber et al., 2013; FAOSTAT, 2014; World Bank Group Report, 2016). From the agricultural sector, methane is generated via enteric and fermentation. manure fertilizers management, rice cultivation, and other agricultural activities. Methane emission from waste include those from landfilling of solid waste, waste water, waste combustion and the use of solvent and other products (Karakurt et al., 2012; Gerber et al., 2013; FAOSTAT, 2014; World Bank Group Report, 2016).

This review examines the contribution of the agricultural sector to CH<sub>4</sub> emissions, with particular reference to enteric fermentation, eructation and their mitigation strategies.

#### METHANE EMISSIONS FROM THE AGRICULTURAL SECTOR

Figure 1 shows the contribution of the different subsectors to global CH<sub>4</sub> emissions. Methane emissions from agricultural sources have increased from 4,656 Mt CO<sub>2</sub>-eq in 2000 to 5,382 Mt CO<sub>2</sub>eq in 2012 (World Bank Group Report, 2016; FAOSTAT, 2014). This value makes the agricultural sector to be the biggest

emitter that is responsible for the majority of methane emissions from anthropogenic sources. In other words, 50.63% of anthropogenic methane emissions are released as a result of agricultural activities. Sources causing methane emissions in agriculture sector as indicated in Figure 1 are enteric fermentation (40 %), manure management (25%), fertilizers (13%), rice cultivation (10%) and other agricultural sources (12%; Gerber *et al.*, 2013).



#### **Figure 1: Agriculture emissions by subsector** Source: Gerber *et al.* (2013).

Enteric fermentation deals with the fermentation of feedstuffs by microbes in an animal's digestive system (Moss *et al.*, 2000). As a result of this process, methane is released by eructation. Domesticated ruminants such as cattle, buffalos, sheep, goats, and camels account for the majority of methane emissions in this sector (Wittenberg, 2010; Gworgwor *et al.*, 2006). Other domesticated non-ruminants such as

swine and horses also produce methane as a by-product of enteric fermentation, but emissions per animal species vary significantly. Figure shows 2 the contribution of different farm animals' species to enteric methane production. Total methane emissions from sector are related to livestock type and population and quality and type of feed (Mangino and Peterson, 2010; EPA, 2006).



#### Figure 2: Global estimated emissions by species.

Source: Gerber et al. (2013).

When manure is stored or treated in liquid systems such as lagoons, ponds or pits, anaerobic conditions will be developed and methane emissions result from the decomposition process (Steed and Hashimoto, 1994). The amount of methane from manure varies with respect to the types of animals and diets, composition of manure, moisture conditions and ambient temperature for storage (Karakurt et al., 2012). The decomposition of organic materials in an environment without oxygen in flooded rice fields also lead to the release of methane. When the rice fields are flooded, decomposition of organic materials gradually consumes the oxygen which is available in soil and water. Once the oxygen consumed. in the environment is methanogenic bacteria release methane. The amount of methane from rice paddies is under the control of several factors including the quantity of organic materials and water management (IPCC, 2009; Anand et al., 2005). Other agricultural sources causing methane emissions include burning of biomass, savanna burning, burning of agricultural residues, cultivation of organic soils and burning of forest clearings (EPA, 2006). This review will dwell on enteric methane production and mitigation strategies.

#### MECHANISM OF ENTERIC METHANE PRODUCTION *Methanogens:*

Enteric methane (87 - 90%) is produced in rumen, the remainder being released from fermentation in the large intestine (Lascano and Cárdenas, 2010). Methane production in the rumen occurs due to the presence of organisms belonging to the kingdom *Archaea* (Van Soest, 1982). They are considered to be an ancient and unique group of organisms which are the strictest of anaerobes and inhabit some of

the harshest and primitive most environments on earth (Hook et al., 2010). One of these unique environments happens to be the rumen. Methanogens are found in a variety of other ecosystems including swamps, heat vents on the ocean floor, rice fields, and in the gut of termites (Hook et these al.. 2010), and sources are quantitatively important to worldwide methane emissions (Pesta, 2015).

Seven orders of methanogens have taxonomists; classified been bv Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, Methanocellales, Methanopyrales, and most discovered. recently Methanomassiliicoccales (Pesta, 2015; Borrel et al., 2013). Methanogens in the order Methanopyrales thrive on conditions greater than 60° C and therefore are not present in ruminants. Members of the order Methanococcales are commonly isolated around submarine hydrothermal vents.

Hook et al. (2010) in their review the identification reported of orders Methanobacteriales, Methanomicrobiales, and Methanosarcinales in the gastrointestinal tract of ruminants. Species within the order Methanobacteriales have been the most commonly identified methanogens in the rumen (Hook et al., 2010). Wright et al. (2006) reported the presence of clones from Methanomicrobiales in ovine rumen fluid while Whitford et al. (2001) reported that two members of the order Methanobacteriales (Methanobrevibacter ruminantium and *Methanosphaera* stadtmanae) were the largest groups of methanogens found in the rumen of dairy cattle fed total ration. а mixed Methanobrevibacter ruminantium almost exclusively utilizes CO<sub>2</sub> reduced with H<sub>2</sub> as a source of electrons, along with formate,

which is degraded to  $CO_2$  and  $H_2$  (Pesta, 2015; Hook *et al.*, 2010).

The order of Methanosarcinales, which have cytochromes that act in the oxidation of methyl groups to CO<sub>2</sub>, are slower growing than the other 2 orders of methanogens in the rumen but are the most versatile methanogens, as they are capable of utilizing four different pathways for methanogenesis (Borrel, et al., 2013). Some examples of these organisms include Methanosarcina barkeri and Methanosarcina mazeii which can use a wider variety of substrates including acetate, methylamines, and methanol (Hook et al., 2010). Nicholson et al. (2007) found Methanosarcinales species in fluid from cattle and sheep.

# Biochemistry of Methane Production in the Rumen:

Kim and Gadd (2008) described the grouping of methanogenesis pathways according to the electron donors used; hydrogenotrophic, methylotrophic, and aceticlastic. Hydrogenotropic is the most common pathway used by methanogens found in the reticulorumen of ruminant livestock (Hook et al., 2010). There is also evidence for a fourth pathway which is a methylotrophic specific type of methanogenesis: H2-dependent methylotrophic methanogenesis (Welander and Metcalf, 2005). Regardless of grouping, CO<sub>2</sub> is the major electron acceptor in methanogenesis. A variety of electron donors are utilized by methanogens, including H<sub>2</sub>, formate, methanol, acetate, methylamines, and carbon monoxide, although the of majority known methanogens grow when H<sub>2</sub> is used as the electron donor (Kim and Gadd, 2008). Multiple unique cofactors are required for methanogens to function, the most prevalent of which are coenzyme F420, coenzyme B (7-mercaptoheptanoylthreonine phosphate), coenzyme M (2-mercaptoethanesulfonate), methanofuran, and 5.6.7.8tetrahydromethanopterin (Deppenmeier, 2002).

Reduction of CO<sub>2</sub> to CH<sub>4</sub> is an important component of the rumen ecosystem due to its role as a recycler of NAD<sup>+</sup> (Russell, 2002). Two molecules of NADH are produced when a molecule of glucose is fermented to pyruvate in the rumen via the Embden-Meyerhoff/glycolytic pathway. Under aerobic conditions, an additional 8 moles of NADH are produced through the citric acid cycle, and are reoxidized to NAD<sup>+</sup> through the electron transport chain. In anaerobic fermentation, oxygen is not available to be an electron acceptor and a replacement must be used for the terminal end of the electron transport electron acceptor during chain. The methanogenesis is H+, which serves an important role by receiving electrons from NADH, thus regenerating NAD+ and producing H<sub>2</sub> (Russell, 2002). Methanogens use this  $H_2$  as an electron donor to produce CH<sub>4</sub>. In the absence of methanogens, interspecies hydrogen transfer is accomplished through bacterial fermentation which produces lactate, acetate, ethanol, succinate, or propionate (Wolin, 1982). Through this process, it becomes clear that energetic losses due to methane production are due to a loss of H+, not a loss of carbon.

#### METHANE STRATEGIES

MITIGATION

The methane mitigation strategies that will be reviewed in this paper include animal genetic selection and breeding as well as improved dietary provisions.

# Animal Genetic Selection and Breeding

Livestock production has been intensified through better breeding and feeding programmes to decrease global greenhouse gas emissions (Martin *et al.*, 2009). Animal production efficiency has been increased by selection of animals with improved genetic merit (the ratio of an animal's performance with the group

average). Factors responsible for variability between animals are the rate of passage, salivation rate, feeding rate, drinking rate, volumes. microbial activity. rumen fermentation conditions, seasonal variation and grazing behavior (Mirzaei-Aghsaghali et al., 2015; Pinares-Patiño et al., 2003). In dairy cows, body weight, milk yield, and type of roughage influence CH<sub>4</sub> production. Animals with high genetic merits produce 7 - 27% less methane, as a percentage of gross energy (GE) intakes (Boadi and Wittenberg, 2002). Nkrumah et al. (2006) reported that low Residual Feed Intake (RFI) animals (i.e., efficient animals) emit up to 28% less methane than high RFI counterparts. Cattle that eat less than their peers of equivalent live weight and average daily gain have a low residual feed intake and are more feed efficient RFI is a measure of net feed efficiency. RFI is a moderately heritable trait (Herd and Arthur, 2009).

Ulyatt et al. (2002) reported that 71 -95% of variation in methane emission from dairy cows and breeding ewes grazing rvegrass/white clover pasture in New Zealand between days was attributable to animals. Pinares-Patino et al. (2003) also reported that about 85 % of the variation in daily CH<sub>4</sub> production (g/day) from sheep grazing temperate pastures was due to variation between animals. This degree of variability reported is a strong indication that breeding animals with low methane emissions but with uncompromised performance will go a long way in mitigating enteric methane emissions (Buddle et al., 2011).

#### Improved Dietary Provisions

Some dietary or nutritional strategies to mitigate methane production have been identified and adopted in dairy animals and these includes the addition of ionophores, dietary Lipids supplementation, the use of high-quality forages or *preserved forages (Silage)*, and increased use of grains and concentrates; addition of modern feed additives like probiotics, acetogens, bacteriocins, archaeal viruses, organic acids, plant extracts (e.g., essential oils) to the diet (Boadi *et al.*, 2004).

### Dietary Lipids Supplementation:

Vegetables and animal lipids were originally used in ruminant rations to increase their energy density. However, lipids are also considered useful today to reduce enteric methanogenesis (Brask et al. 2013: Beauchemin et al.. 2007). Supplemental fat is the most commonly studied dietary component that can act as an  $H^+$ sink to reduce methanogenesis. However, there are several modes of action by which fats can inhibit CH<sub>4</sub> production: by directly inhibiting methanogens and protozoa, by directing H<sup>+</sup> toward biohydrogenation of unsaturated fats, bv increasing propionate production or likely by a combination of bio-hydrogenation of unsaturated fatty acids and direct inhibition of activities of different microbes including methanogens (Hook 2010: et al.. Beauchemin et al., 2007). Factors that may account for varying effects of lipids on methane abatement are the ruminant species, experimental diet, and the type of lipid used (Hook et al., 2010)

A meta-analysis by Grainger and Beauchemin (2011) found that in diets with 8% or less dietary fat, a 1% increase in dietary fat would result in a decrease of 1g CH4/kg DMI. Beauchemin *et al.* (2008) observed a 5.6% methane reduction per percentage unit of lipid added to the diet of cattle and sheep. Comparison of the effects of different fatty acids revealed that lauric, myristic and linoleic acids were the most potent reducers of methanogenesis (Zhou *et al.*, 2013; Ding *et al.*, 2012; Jordan *et al.*, 2006).

The potential of essential oils as additive to manipulate rumen fermentation and decrease methane emissions has been extensively investigated and reviewed (Benchaar and Greathead 2011; Calsamiglia *et al.*, 2007). A wide range of essential oils (derived from garlic (*Allium sativum*), eucalyptus (*Eucalyptus globules*) thyme, neem (*Azadirachta indica*), oregano, cinnamon, rhubarb, frangula) has been shown to decrease methane production *in vitro* by 55.8% (Sirohi *et al.*, 2013).

The addition of different oil (soya, coconut, canola, rapeseed, etc.) to ruminant diets have been shown to reduce methane production between 19% and 62% in sheep (Ding et al., 2012), beef cattle (Jordan et al., 2006) and dairy cows (Brask et al., 2013; Odongo et al., 2007). Refined soy oil based diet fed to beef bulls reduced methane by 39% (Jordan et al., 2006). Martin et al. (2008) reported a 55.8 - 64% reduction in grams of methane per day by lactating dairy cows fed linseed oil supplemented at a level of 5% of DM. Beauchemin and McGinn (2006) reported a 21% decrease in CH<sub>4</sub> as a percentage of GE intakes and a 15% decrease in DM digestibility when they fed canola oil. Differences in performance were observed in a study by Fiorentini et al. (2014), in which steers were fed whole soybean, linseed oil, or palm oil as sources of fat that differed in saturation. Linseed oil and whole soybeans, rich in unsaturated fatty acids, decreased CH<sub>4</sub> by 1.1g/kg DMI for every 10g of fat consumed. Palm oil however, a saturated fatty acid, decreased CH<sub>4</sub> by 1.8g/kg DMI for every 10g of fat consumed and severely decreased DMI, average daily gain (ADG) and feed conversion ratio. The authors attributed the additional CH<sub>4</sub>-mitigating effect of palm oil compared to unsaturated fats to the greater content of medium chain fatty acids in palm oil, which can be toxic to methanogenic archaea.

However, the inclusion of lipids at levels above 6 - 7% of dry matter intake can affect palatability; reduce feed intake and fibre digestibility, resulting in lower performance, lower milk yield and composition or daily gain (Patra, 2012; Odongo *et al.*, 2007; Jordan *et al.*, 2006). Thus, when oils are used, decreases in CH<sub>4</sub> must be balanced against decreased digestibility, DMI and animal performance.

## Dietary Lipids By-Products:

High-oil by-products from the biofuel industries - dry distillers grains (DDG) or wet distillers grains (WDG) alone or with solubles (DDGS and WDGS, respectively) and mechanically extracted oilseed meals can naturally serve as CH4 mitigating feed, if included in the diet to decrease feed cost (Hristov et al., 2013). McGinn et al. (2009), for example, reported up to 24% less CH<sub>4</sub> emissions when DDG replaced barley grain in the back grounding diet of beef cattle by supplementing an additional 3% lipid to the dietary DM. However, the effects of distillers' grains on CH<sub>4</sub> production are not consistent and might depend on the rest of the diet. Hales et al. (2013) fed diets containing 0 to 45% WDGS (substituting steam-flaked corn) to Jersey steers and observed a linear increase in CH<sub>4</sub> emission per unit of DMI (up to 64% increase with the highest inclusion rate), due primarily to increased NDF intake, although the ether extract (EE) content of the diet increased from 5.9 to 8.3%. High-oil byproduct feeds might have the same suppressive effect on feed intake as free lipids, so caution must be exercised to prevent negative effects on animal productivity or milk fat depression in lactating cows (Schingoethe et al., 2009).

## Inclusions of Concentrates

It is well established that increasing the level of concentrate in the diet leads to a reduction in CH<sub>4</sub> emissions as a proportion of energy intake or expressed by unit of animal product (milk and meat) if production remains the same or is increased. Concentrate feeding can reduce methane output by reducing the protozoal population (Van Soest, 1982) or by changing rumen volatile fatty acids (VFAs) concentrations to

favour the production of more propionate and less acetate.

Decreases in CH<sub>4</sub> emissions per kilogram of animal product (19.26 and 16.02g of CH<sub>4</sub>/kg of fat-corrected milk) have been reported (Lovett et al., 2005) with an increase in the proportion of concentrate the diet (Aguerre et al., in 2011: Beauchemin and McGinn, 2006). However, increasing the concentrate proportion in the diet above certain levels, however, might have a negative effect on fiber digestibility (Ferraretto et al., 2013;), which, in addition to a potential loss of production, could result in increased concentration of fermentable OM in manure and perhaps increased CH<sub>4</sub> emissions from stored manure (Hristov et al., 2013). Increased levels of concentrates may also result in health problems e.g. acidosis.

# Forage type and Quality

The CH<sub>4</sub> emissions (g/kg DMI) from animals fed forage legumes is usually (McCaughey *et al.*, 1999), but not always (Hammond *et al.*, 2011) lower than emissions from animals fed predominantly grasses. Decreased CH<sub>4</sub> production of 10 – 21% per unit of product has been reported for highly digestible forages such as lucerne (Benchaar *et al.*, 2001; McCaughey *et al.*, 1999). Factors responsible for lower emissions for animals fed legumes are often explained by the presence of condensed tannins, lower fibre content, higher DMI and faster rate of passage from the rumen.

Forage quality, especially its digestibility also affects the CH<sub>4</sub> production potential of the forage. The effects of forage quality on CH<sub>4</sub> emissions are often contradictory (Hart et al., 2009; Molano and Clark, 2008; Pinares-Patiño et al., 2003). Feeds with higher digestibility recorded increased DMI but depressed CH<sub>4</sub> produced per unit of feed consumed (Hammond et al., 2009, 2013), whereas, increased intake of less-digestible poor-quality, preserved forages has little effect on CH<sub>4</sub> production when expressed on a DMI basis (Johnson and Johnson, 1995). Forages from temperate rangelands (C3 grasses) emit less methane (17%) per unit of intake than grasses from tropical regions which are C4 grasses (Ulyatt *et al.*, 2002). Forage maturity at the time of harvest also influence CH<sub>4</sub> emissions, with increased CH<sub>4</sub> emission reported (5 - 6.5%). with forage maturity (Chaves *et al.*, 2006).

# Using Preserved Forages: Silage

Forage preservation and processing also affect enteric  $CH_4$ production. Methanogenesis tends to be lower when forages are ensiled than when they are preserved as hay, and when they are finely ground or pelleted than when coarsely chopped (Beauchemin *et al.*, 2008). Benchaar et al. (2001) reported that total methane production was depressed (-33%)by the utilization of alfalfa silage instead of alfalfa hay. They also reported that fractions of GE intake and DE lost as methane were also lower (-32 and -28%, respectively)with alfalfa silage than with alfalfa hay. Varga et al. (1985) reported a decrease in methane production from cattle consuming alfalfa silage compared to orchard grass silage. Some studies have indicated reduced CH<sub>4</sub> production with corn vs. grass silages (Doreau et al., 2012). Dewhurst (2012) gave a comprehensive overview of the various aspects of feeding corn versus legume versus grass silages for lactating dairy cows. The author concluded that the lower fiber content and higher passage rates of legumes appeared to decrease CH<sub>4</sub> production compared with grasses, which was reported in earlier studies (McCaughey et al., 1999).

## CONCLUSIONS

• Sub-sectors contributing to the global agricultural CH<sub>4</sub> emissions include enteric fermentation, manure management, fertilizers application and rice cultivation, with enteric

fermentation contributing 40%. In order to regenerate NAD<sup>+</sup>, the methanogens utilized H<sup>+</sup> as an electron acceptor of H<sup>+</sup> in NADH to produce H<sub>2</sub>. Methanogens then used the H<sub>2</sub> as an electron donor to reduce  $CO_2$  to CH<sub>4</sub>. Thus, to reduce global warming as well as improve animal productivity, animals with high genetic potentials should be selected.

• Inclusion of lipids and lipid byproducts in the diet at optimum level, use of highly digestibility forages, optimum inclusion of concentrate in the diet and the use of legumes and preserved forages like silage will also go a long way in the mitigation of enteric methane emissions.

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