Can Inoculation of Silage with a Ferulic Acid Esterase-producing Inoculant reduce Enteric Methane Emissions in Ghana?

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ABSTRACT

Methane (CH_4) is a potent greenhouse gas accounting for 25 times the capacity of CO_2 in causing global warming. Enteric CH_4 emission from domestic ruminant production is greater in the tropics where more forage is fed than in temperate regions where concentrates can form more than 85% of the diet. Inoculation of silage with a fibrolytic inoculant improves fibre digestibility. Increased ruminal fibre digestion increases ruminal nutrient digestion, fermentable substrates and passage rate. This reduces the time available for methanogenesis to occur in the rumen. A glucogenic pattern of rumen fermentation associated with feeding silage inoculated with ferulic acid esterase-producing inoculant increases propionic acid in the rumen. Formation of ruminal propionic acid serves as a sink for H_2 that would otherwise be used for methanogenesis. At a lower ruminal pH, methanogens also lose their ability to use H_2. Efficient utilization of feed can therefore reduce enteric CH_4 output. Other benefits of inoculating silage with fibrolytic silage inoculants include improvements in aerobic stability which also reduces emission of obnoxious gases such as nitric oxide, which reacts with atmospheric oxygen to form nitrogen dioxide. Abatement of CH_4 emission by ensiling with ferulic acid esterase-producing inoculants may therefore represent a less expensive and most practical management strategy for reducing enteric CH_4 emission in Ghana.

Keywords: *Enteric methane, ferulic acid esterase-producing inoculant, fibre digestibility*

INTRODUCTION

In Ghana, enteric methane (CH_4) emission from livestock is rated second only to agricultural soils (artificial N fertilizers) as the leading emitter of greenhouse gases (UNEP, 2013). This is apparently because the main feed resource for ruminant production in Ghana is wild natural forages whose digestion has been associated with increased enteric CH_4 formation *in vitro* (Meale *et al.*, 2012). Methanogenesis in the rumen does not only represent significant diversion of dietary energy away from growth but also CH_4 is a potent greenhouse gas accounting for 25 times the capacity of CO_2 in causing global warming (Hook *et al.*, 2010).

Methane output per unit feed intake or per unit of product formed can be reduced by improving the efficiency of feed utilization. Efficient utilization of forages by ruminants is however limited by poor digestibility due to deposition of phenolic compounds such as *p*-coumaric and ferulic acids (FA) that inhibit the growth of major fibrolytic bacteria and depress fibre digestibility in the rumen (Akin *et al.*, 1988).

As forage quality is a function of ruminal fibre digestibility, ruminal CH_4
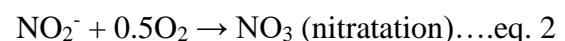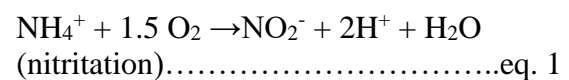
output is strongly related to forage quality and NDF digestibility (Hook *et al.,* 2010). Shifting ruminal fermentation of fibre towards higher acetate production increases CH_4 output by providing methyl groups for methanogenesis whereas a shift to higher ruminal propionate production decreases it because the pathway for propionate formation involves the utilization of H_2 (Moss *et al.,* 2000). Options for reducing enteric emission of CH_4 include reducing livestock numbers, using genetically efficient animals, directly altering rumen microbial populations and improving diet quality. However, improvements in feed quality appears to be the most viable and reasonable option in resource-constrained production systems as all other strategies have so far either not been effective or have resulted in adverse effects on feed intake (Hook *et al.,* 2010). Inoculation of silages with FA esterase-producing inoculants containing *Lactobacillus buchneri* has previously improved fermentation and NDF digestibility of silages (Nsereko *et al.,* 2004; Kang *et al.,* 2009; Addah *et al.,* 2012a). Improvement in fermentation and aerobic stability also reduces the emission of obnoxious gases such as nitrate (NO_3), nitrite (NO_2) and ammonia which have a greenhouse effect on the environment (Oude Elferink *et al.,* 2000). Emissions from silage production have shown that there are numerous (more than 50) volatile organic compounds that can contribute to ozone aperture formation in the troposphere (Chung *et al.,* 2009; Howard *et al.,* 2010) but production of some volatile organic compounds may be reduced by bacterial inoculation of silages (Hafner *et al.,* 2013).

Earlier studies have suggested that even though FA reduces NDF digestibility, it also contrarily reduces CH_4 production *in vitro* (Martin, 1988). However, greater NDF digestibility of cattle fed inoculated silage has been associated with increased ruminal propionate and decreased acetate concentrations (Keady *et al.,* 1994; Addah *et al.*, 2011b). This pattern of fermentation in the rumen involves the utilization of H_2

thereby reducing H_2 availability for methanogenesis in the rumen. Increased NDF degradability increases ruminal fermentable substrates, digestion and passage rates and lowers ruminal pH thereby reducing the time available for methanogenesis to occur in the rumen (Hook *et al.,* 2010; Moss *et al.,* 2000). At lower ruminal pH, methanogens also lose their ability to use H_2 independent of the process of propionate formation (Moss *et al.,* 2000). This review examined the possibility of ensiling forages with fibrolytic inoculants to reduce CH_4 output in Ghana.

Emissions from Silage production

Silage fermentation by itself produces obnoxious gases that can contribute to holes in the ozone and inoculation of the silage with bacterial inoculants capable enhancing the fermentation process and improving aerobic stability should help abate the release of obnoxious gases from silage that contribute to apertures in the ozone layer (Hafner *et al.,* 2013). The most important greenhouse gases are carbon dioxide, CH4 and nitrous oxide. Nitrates present in silage crops are converted to nitrites through the fermentation process; they, in turn, react with organic acids in the silage to form nitrous acid (HNO_3). As the temperature of the silage increases during fermentation, the nitrous acid decomposes to form a mixture of oxides of nitrogen, which can include NO, NO_2, nitrogen trioxide (N_2O_3), nitrogen tetroxide (N_2O_4) and other oxides in lesser amounts (Oude Elferink *et al.,* 2000). Nitrification is a two-step process (eq. 1 and 2), where ammonium nitrogen (NH_4^+) is oxidized to nitrite (NO_2^-) which is then oxidized to nitrate (NO_3^-). Our studies with silage inoculants have consistently demonstrated that inoculation reduces silage temperature upon aerobic exposure (Addah *et al.* 2011ab; 2012ab).

$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + 2H^+ + H_2O$ (nitritation)…………………………..eq. 1

$NO_2^- + 0.5O_2 \rightarrow NO_3$ (nitratation)….eq. 2

Ensiling strategies capable of rapidly reducing silage pH could therefore help to reduce emissions of obnoxious gases from silage fermentation. In a series of studies, we observed a rapid decline in silage pH when silage was inoculated with homolactic (Addah *et al.,* 2011ab) and heterolactic (Addah *et al.,* 2012ab) silage inoculants. The production of ammonia in silage and subsequently of nitrates and nitrites occurs at higher pH. It is well known that nitrification rate slows down at a pH below 7.0 and it ceases at around pH 6.0 (Yoon, 2011). The rate and extent of pH decline is faster and lowest, respectively, for inoculated than uninoculated silages (Addah *et al.,* 2011b). Inoculants that induce rapid decline in pH should potentially reduce the rates of oxidation of ammonia-N into nitrites and nitrates. Ammonia-N concentration, was lower in control compared to inoculated corn silage when a homofermentative inoculant was used (Addah *et al.,* 2011b) but when a fibrolytic heterofermentative inoculant was subsequently used for barley silage under farm conditions, inoculation reduced ammonia N concentration by 25% (Addah *et al.,* 2012b).

Enteric emissions

Excess H_2 in the rumen must be removed for continued fermentation and microbial growth to occur. The removal of H_2 occurs through the activity of methanogenic Archaea, which reduce carbon dioxide with H_2 to yield CH_4 and water (eq. 3). A symbiotic relationship involving interspecies H_2 transfer between methanogens and other microorganisms, especially protozoa exist in the rumen. Ruminal microorganisms such as protozoa, fungi and some bacteria produce H_2 as one of their end-products of fermentation yet H_2 does not accumulate in the rumen because it is immediately used by other bacteria through interspecies H_2 transfer. The stoichiometry of anaerobic H_2 production and utilization is shown in Table 2.

Ruminal protozoa depend on a H_2 evolving fermentation to provide H_2 for methanogenesis, and protozoa in turn, benefit from H_2 removal by methanogens since H_2 is inhibitory to their metabolism if not removed. In the bovine rumen, *Methanobrevibacter ruminantium* was identified as the largest group of methanogens followed by *Methanosphaera stadtmanae*. Common bovine protozoa and methanogens involved in this interspecies H_2 transfer include protozoa such as *Entodinium, Polyplastron*, *Epidinium* and *Ophryoscolex* and methanogens such as the orders *Methanobacteriales* and *Methanomicrobiales* (Sharp *et al.,* 1998). Anaerobic fungi, such as *Neocallimastix frontalis,* have also been found to have a relationship with methanogens involved in interspecies H_2 transfer whereby the fungi's enzymatic activity is increased and metabolism is shifted towards acetate production (Mountfort *et al.,* 1982). Volatile fatty acids (VFA) are not common substrates for methanogenesis because their conversion into CO_2 and H_2 is a lengthy process (Hobson and Stewart, 1997). Therefore, methanogens depend on CO_2 and H_2 produced by the fermentation of carbohydrates into VFAs (Hungate *et al.*, 1970).

Methane emissions from ruminant livestock have increased fivefold over the last century (Johnson *et al.,* 2000) and now constitute ~15% of global CH_4 emissions (McAllister *et al.,* 1996). Globally, the contribution of enteric fermentation to the rate of CH_4 emission per annum is reported to be highest among all agricultural activities (Fig. 1). Enteric CH_4 is produced primarily from microbial activity in the rumen (90%) with only a minor contribution from the large intestine (10%). The majority of CH_4 produced is therefore released through the mouth and nostrils (Murray *et al.,* 1976).

Methane production represents an energy loss to ruminants of around 3-9% of gross energy intake. It has been estimated that 10-30% of digestible OM can be
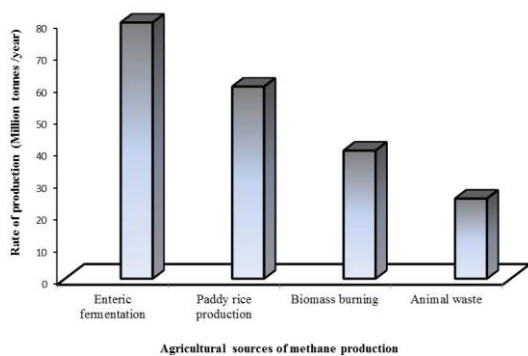
Fig. 1: Rates of emission of CH$_4$ from agricultural sources (Data from Watson *et al.,* 1992)

digested in the hind gut. The anaerobic bacteria in the hindgut are not very different from those found in the rumen (Julliand, 1992). However, protozoa are absent from the hindgut of ruminants and significant methanogenic fermentation in the hindgut is less likely hence not much CH$_4$ is formed in the hindgut.

Feeding forages at maintenance level resulted in CH$_4$ energy loss of 6-7% of gross energy intake compared to 2–3% when high-grain concentrates (> 90% DM) were fed *ad libitum* (Johnson and Johnson, 1995). This is because digestion of concentrates in the rumen shifts fermentation pattern towards greater formation of propionate and lower pH, making the ruminal ecology unsuitable for methanogenic activity.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \dots\dots\dots (eq.3)$$
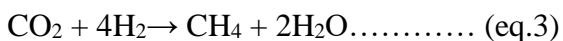
Methane mitigation strategies

Options for reducing enteric emission of CH$_4$ include reducing livestock numbers, using genetically efficient animals, improving diet quality and altering rumen microbial populations. However, improvements in feed quality appears to be the most viable and reasonable option in resource-constrained production systems as all other strategies have so far either not been effective or have resulted in adverse effects on feed intake (Hook *et al.,* 2010). Dietary mitigation strategies to reduce methanogenesis in the rumen include i) the

direct inhibition of methanogens, ii), reducing the production of H$_2$ in the rumen or iii) providing alternative sinks for the disposal of H$_2$ (Beauchemin *et al.,* 2009). Direct effects on methanogens include the use of antimicrobial agents such as essential oils, tannins, saponins and monensin. Indirect mitigation strategies include defaunation, supplementation with organic acids (e.g. fumarate and malate) and unsaturated fatty acids to serve as H$_2$ sinks, methanogen vaccine and forage preservation and processing to shift ruminal fermentation patterns towards lower CH$_4$ production. However, besides the latter strategy, all other strategies have so far been either ineffective or have resulted in adverse effects on feed intake (Hook *et al.,* 2010). A reduction in CH$_4$ production is expected when the residence time of feed in the rumen is reduced since ruminal digestion decreases and methanogenic bacteria are less able to compete in such conditions. Methane emissions from livestock fed legumes should therefore be lower than emissions from livestock fed grass-based forages. Okine *et al.* (1989) observed a 30% decline in CH$_4$ production when the ruminal passage rate of liquid and solid phase increased by 54-68%. In that study, retention time was shown to explain 28% of the variation in CH$_4$ emissions. As the level of feed intake increases, passage rate increases and time for methanogenesis decreases as well. The major effect of feeding level is therefore explained by its consequences on passage of feed particles out of the rumen (Owens and Goetsch, 1986). Measures that increase ruminal solubility of forage fibre are expected to increase intake and passage rate and thereby reduce enteric CH$_4$ output. In our earlier studies, we found that inoculation of silage with a fibrolytic silage inoculant resulted in a glucogenic pattern of fermentation, increasing the proportion of ruminal propionic acid apparently due to partial solubility of fibre in the silo during ensiling (Addah *et al.,* 2012b). We have particularly observed that ruminal propionate and total

volatile acid (VFA) production also increased and feed utilization efficiency improved when beef cattle were fed diets containing 75% DM of barley silage inoculated with a ferulic acid esterase-producing inoculant (Addah *et al.,* 2011b; 2012b). This indicates that more CH_4 will be produced from less efficient animals especially when fed poor quality forages. Abatement of CH_4 emission by ensiling may therefore represent a less expensive and most practical strategy for ruminants in tropical Africa where forage is the main source energy for ruminants. Preliminary studies in New Zealand have indicated that silage could reduce CH_4 output of dairy cattle compared to pasture (Woodward *et al.,* 2001). Other previous studies have also confirmed that certain lactic acid producing in some silage inoculants survive within the rumen environment and may modify rumen fermentation patterns (Weinberg *et al.,* 2003; 2004) towards a higher propionate: acetate ratio thereby providing alternate sinks for H_2 as well as potentially improving DM and NDF digestibility (Weinberg *et al.,* 2007). Boadi and Wittenberg (2002) have shown that steers grazing early-season pasture produced up to 45% less CH_4 than those grazing in the mid- and late- season. This suggests that as the deposition of phenolics into the cell wall increases with maturity, the proportion of rumen fermentable forage fibre and passage decreases, and CH_4 output increases. The benefits of rapid decline in silage pH, improvements in aerobic stability, shifts towards glucogenic pattern of rumen fermentation and improvements in feed efficiency associated with bacterial inoculation of silages (Addah *et al.,* 2011ab; 2012ab) suggest that silage inoculation has the potential to mitigate enteric CH_4 emission as well as obnoxious gases released from silage fermentation and/or decomposition.

CONCLUSIONS AND IMPLICATIONS

Strategies to reduce CH_4 emissions have often been based on supplementation with methanogen inhibitors. Such strategies have not only been ineffective because of the development of resistant strains of methanogens over time but have also been associated with extra cost and reduced feed intake. Improvements in silage fermentation, aerobic stability and ruminal fibre digestibility, and accompanying shifts in ruminal fermentation patterns, through inoculation of silages with ferulic acid esterase-producing inoculant could reduce enteric CH_4 emission by ruminants in Ghana.

The survival of some strains of lactic acid producing bacteria in modern silage inoculants within the rumen and their ability to modify rumen fermentation patterns and potentially improve DM and NDF digestibility offers an opportunity for modifying rumen fermentation towards reduction of enteric CH_4 emission. Research efforts should be directed at strategies that are easily adaptable by livestock farmers in resource-poor farming systems if reductions in CH_4 production from agricultural activities are to be sustainable in developing countries such as Ghana.

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Table 1. Fibre composition (g/kg DM), CH4 production (mg/g digested DM), and volatile fatty acid (mol/100 mol of total) and ammonia (mM) accumulation of different forage species from Northern Ghana, after 24 h fermentation *in vitro* (Meale *et al.*, 2012)

Forages	Fibre		CH4	Ammonia	volatile fatty acids	
	NDF	Lignin			Acetate	Propionate
Leguminous shrubs						
Cajanus cajan	478	173	9.6	22.5	66.0	19.3
Gliricidia sepium	427	125	8.0	-4.4	66.2	21.7
Leucaena leucocephala	377	95	7.3	17.4	67.0	20.0
Non-leguminous shrubs						
Moringa oleifera	249	53	6.4	0.4	61.2	20.7
Vitellaria paradoxa	89	298	0.4	2.2	62.7	24.2
Grasses						
Andropodon gayanus	72	82	15.6	6.3	66.1	20.4
Brachiaria ruziziensis	87	64	12.1	10.6	64.8	22.6
Pennisetum purpureum	87	56	11.6	11.6	65.8	20.9

Table 2. Characteristics common methanogens in farm animals

Methanogen	Common characteristics	Substrates	Common farm animal	Reference
Methanobrevibacter ruminantium	Rod shape; variable motility Common in low-energy diets	H_2, CO_2 and formate	Lactating dairy cattle; sheep	1; 2
Methanobacterium formicicum	Rod or filament shape; immotile	H_2, CO_2 and formate	Grazing cattle	3
Methanomicrobium mobile	Rod shape; motile	H_2, CO_2, and formate	Lactating dairy cattle; sheep	2; 3
Methanosarcina barkeri	Coccoid shape; immotile cytochromes (membrane-bound electron carriers; for oxidation of methyl groups to CO_2)	H_2, CO_2, acetate, methylamines and methanol	Absent in grazing cattle but present in indoor cattle	3

[1]Whitford *et al*. (2001); [2]Yanagita *et al*. (2000); [3]Jarvis *et al*. (2000)

Table 3: Hydrogen production and utilization in the rumen

Hydrogen-producing reactions	Hydrogen-utilizing reactions
Glucose $\rightarrow$ 2 pyruvate + 4H	Pyruvate + 4H$\rightarrow$ propionate (C3) + H_2O
Pyruvate + H_2O $\rightarrow$ acetate (C2) + CO_2 + 2H	Acetate (2 C2) + 4H$\rightarrow$ butyrate (C4) + 2H_2O
	CO_2 + 8H$\rightarrow$ methane (CH_4) + 2H_2O