Animal Husbandry, Dairy and Veterinary Science

Review Article

ISSN: 2513-9304

The role ensiled forage has on methane production in the rumen

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Abstract

Methane emitted by ruminants is not only a significant greenhouse gas but a loss in productivity because of the energy lost from the animal. Ensiled forage is fundamental in the nutrition of housed ruminants. Therefore a review of how ensiled forages influences enteric methane provides an understanding of what mitigation measure are achievable by the producer and what further research is required. Inclusion of forage maize silage in diets has consistently shown 10-20% reductions in enteric methane reductions by numerous studies, however the level of reduction is dependent on the maturity of the forage the forage maize silage is replacing. Whereas inclusion of legume silages has been shown to have no significant benefits, even though this forage type has less structural carbohydrates than that of the grass silage it has substituted. Grass swards cut at their immature stage have been shown to reduce enteric methane but best practise of ensiling, silage fermentation and feed out is essential for this benefit to be fulfilled. Inoculants using *Lactobcillus sp.* can assist in doing this and in doing so greater prominance of this mitigation strategy can be given. Going forward the review picks up on further research in areas such as the type of *Lactobaciilli sp.* used as an innoculent as it may enhnce the rumen fermentation process itself; the use of exogenous fibrolytic enzmes in enhncing the ensiled forage digestability and tannin and saponin rich forages. These strategies have been inconsistent in delivering results or are uneconomically viable. However if research can be directed towards understanding how different methanogenic *Archae* operate in the rumen and targetted plant breeding of forages containing bioactive componds, then it may be possible to unlock the potential of future enteric methane mitigation approaches.

Introduction

The impact that livestock production has on the environment was highlighted by FAO (2006) [1] in their 'Long Shadow of Livestock Production' report, with enteric methane (ECH_4) emissions being a key issue. ECH_4 produced by methanogenic *Archaea* in the anaerobic environment of the rumen-reticulum [2] is responsible for approximately 15% of global warming, largely because methane is 25 times more potent than CO_2 as a greenhouse gas [3]. The other downside of ECH_4 emissions is the energy lost to the animal which brings about production inefficiencies that can be anywhere between 2 and 12 % [4].

The review by Knapp *et al.* (2014) [5], looking at the opportunities to reduce methane in dairy production, listed nutrition being at the forefront in achieving this goal. Given ruminants require 50% or more forage in their diet to maintain a healthy and effective functioning rumen [6] and the need to balance out annual forage growth patterns with the requirements of the ruminant by ensiling forages [7], then it can be convincingly argued that silage production is integral in the mitigation of enteric methane. Aspects of silage type and mix; silage quality based on ensiled material; the fermentation process it undergoes; silage inoculants and additives; and novel compounds all play a part in reducing ECH_4 and are covered in the remainder of the report.

Silage type and mixture

The type and mixture of ensiled forage fed will have a direct effect on the microbial population within the rumen that consequently influence the level of methanogenic bacteria proliferation [8]. Therefore, it is necessary to understand how different ensiled forages affect ECH_4.

Forages with a higher amount of dietary starch will favour the amyolytic bacteria population which will result in propionate production so capturing hydrogen in the process and starving the methanogenic bacteria of an essential substrate required for them to operate [9]. In addition, the greater level of propionate in the rumen lowers the rumen pH that is a condition not likened by the methanogenic bacteria, thus the lesser the methanogenic bacteria population the greater the reduction in ECH_4. Whereas if the ensiled forage element is structure fibre based, then cellulolytic bacteria predominate with acetic acid being produced alongside hydrogen and a higher pH environment. The result being conditions that allow the rumen methanogenic bacteria to proliferate and consequently an increase in ECH_4.

Reviews of ECH_4 mitigation in ruminants have documented that ensiled forages with a lesser proportion of structural carbohydrates (cellulose and hemicellulose) will degrade more quickly in the rumen and be digested more readily resulting in lower ECH_4 [2,10]. On this basis it is worthwhile considering ensiled legumes as well as high starch forage crops.

Maize silage compared to grass silage

Based on the above rumen nutritional fundamentals it is not surprising there is a large body of evidence of lessening ECH_4 with maize silage feeding compared to grass silage [11]. An *in-vitro* study by Lengowski *et al.* (2016) [12] examining the differences in rumen

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Key words: *enteric methane, fermentation, forage, quality, ruminant*

Received: October 03, 2018; **Accepted:** October 12, 2018; **Published:** October 16, 2018

microbial population between grass and maize silage, subsequently observed a significant ($P<0.05$) lesser amount of methane produced with maize silage (77.2 ml.day^{-1}) than with grass silage (117 ml.day^{-1}). This 35% decline in CH_4 occurred even though methanogenic bacteria numbers remained the same for both silages. The authors hypothesised the difference arising because of a methanogenic bacterial order change to those of lower methanogenic activity with maize silage; a hypothesis that warrants further examination.

In-vivo studies have also shown similar outcomes. Van Gastelen *et al.* (2015) [13] reported an 11% fall based on dry matter intake (DMI) per kg, in ECH_4 when 100% grass silage diet was replaced with that of 100% maize silage. Hart *et al.* (2015) [14] further substantiated these findings in dairy cows by showing that the replacement of grass silage with maize silage was solely responsible for a significant reduction in ECH_4. Lettat *et al.* (2013) [15] showed a similar fall in methane of 13.5% (kgDMI^{-1}) albeit between a diet of 100% alfalfa forage based to one of 100% maize silage. In contrast, Brask *et al.* (2013) [16] when studying the effect of grass silage maturity compared to maize silage found no significance in ECH_4 produced between young cut ensiled grass versus maize silage in dairy cows when related to organic matter digestion.

Legume silage compared to grass silage

Legume silages are suggested to have the capacity of decreasing ECH_4 compared to grass silages. Legumes are considered more rapidly digested with their smaller proportion of structural carbohydrates and consequently quicker rate of passage through the rumen compared with grasses [17,18]. An actual study to quantify this effect was set out by Hassanat *et al.* (2014) [19] where complete timothy grass silage in the forage part of the dairy cows' diet was replaced in several stages with lucerne. Surprisingly the study suggested a trend of ECH_4 increasing as the timothy silage was replaced with lucerne silage ($P=0.1$). However, when methane was expressed over units of DMI there was no difference and it was concluded that exchanging grass silage with lucerne silage was not a viable strategy in mitigating ECH_4. This corresponded with an aspect of another study's findings which observed no alteration in methane levels when ensiled ryegrass was substituted using either ensiled white and red clover [20].

Silage quality

Literature reports a strong linear decline in ECH_4 with increasing DMI [2,21]. Therefore, enhancing the feed intakes of silages by ensuring quality forage going into the clamp and a quality fermentation process both play an important role in the reduction of ECH_4 produced.

Maturity of the forage

The maturity of the forage is central in the composition of the ensiled material which is fed out to the ruminant. As a plant matures the proportion of cell contents, the highly digestible of the plant, diminishes at the expense of the cell wall [22]. The cell wall proportion, therefore the amount of structural carbohydrates and lignin, in a forage is quantified using neutral detergent fibre (NDF) analysis technique. It follows that with increasing plant maturity, NDF will increase resulting in lower DMI by the animal [23] and greater cellulolytic bacteria proliferation in the rumen. This in turn will provide a ruminal environment with greater levels of hydrogen and less acidic thus favouring protozoa [24]. These conditions benefit the methanogenic bacteria hence an expected increase ECH_4.

Research by Warner *et al.* [25] examined this reasoning in detail by studying the effect of early mature; medium and late mature grass silage fed to 54 Holstein Friesian cows in milk, at a high forage to concentrate ratio (80:20) on ECH_4. The study overall found feed digestibility decreased with increasing grass maturity along with a decline in DMI. Daily ECH_4 on the other hand showed a 6% decline with greater maturity but this was significantly counterbalanced when ECH_4 was expressed on a kg per DMI and fat and protein corrected milk basis showing a decline of 7% and 31% respectively. This allowed the authors to conclude that later matured grass material entering the silo greatly lifts the production of ECH_4. This study substantiated the results obtained by Brask *et al.* [16] in an aspect of the study where ECH_4 in lactating dairy cows was measured between grass silage cut three weeks apart which resulted in a 15% NDF lift in the late cut grass. Consequential daily ECH_4 was the same between both but when examined on a kg DMI basis levels of daily ECH_4 was 6.2% lower for the early compared to the late matured grass silage.

Maize silage maturation on the other hand has been shown to decrease in a linear fashion ($P \leq 0.020$). A study by Hatew *et al* (2016) [26] examined the levels of methane produced in dairy cows for forage maize at different harvest maturity resulting in increasing dry matters (25%, 28%, 32% and 40%). As a result, ECH_4 based on DMI decreased (2.3% to 2%) without the cow performance being hindered. These findings were not surprising given the starch level in maize silage increases with maturity due to the cob proportion increasing. Starch constituency has been seen to increase from 25 to 31% for maize silage dry matters of 24 to 32% respectively, but also resulting too in a fall of NDF (47% to 42%) and ADF (28% to 24%) [27]. This study also goes on to explain that a greater proportion of the maize crop's starch bypasses the rumen and is digested in the small intestine so avoiding any chance of the methanogenic bacteria capturing the energy of this bypass proportion.

Maize silage inclusion in ruminant diets therefore has a significant part to play in reducing enteric methane especially if it is harvested later. However, it must be remembered that the overall impact on the environment must be considered and there is mounting evidence that the benefits of ECH_4 reduction by maize silage is offset by land use change. The annual ploughing for forage maize has been shown to release soil sequestered carbon [28]. Also the need balance out the lower CP in maize silage compared to grass/legume silages by importing protein on to farm, which tends to be soyabean meal, again has a huge carbon footprint with the need of land use change to grow ever greater amounts [29]. Therefore, young mature grass silage becomes of greater importance and the necessity for best ensiling practices given the higher crude protein levels in young grass that makes fermentation more difficult [6].

Silage fermentation

Best practice of silage making involves clamping forages at the optimum dry matter; rapid filling of the silage clamp, expelling oxygen by rolling, immediate sheeting, minimizing undesirable bacteria contamination e.g. *Clostridia* and inoculating with homofermentive bacteria e.g. *Lactobacillus planetarium.* All are necessary to establish rapid acidification resulting in the fermentation process being stable which will not revert to a butyric one [30]. This way greater levels of residual sugars; true protein retained (conversely ammonia N decreased) and butyric acid minimized are key factors in maximizing voluntary intakes [6]. Of course, aerobic stability at feed-out becomes essential with high residual sugars being the ideal substrate for aerobic spoilage organisms e.g. yeasts and molds [31]. By managing these important stages of the overall ensiling process DMI can be lifted, consequently mitigation of ECH_4 on a per kg DMI basis.

The role of silage inoculants

Studies have shown that the use of silage inoculants have a positive response in reducing ECH_4. Not only the application of inoculants but also the use of formic acid in unfavourable harvesting conditions assist due to improved animal dry matter intakes [32]. This together with resulting increased animal productivity means less ECH_4 per unit of DMI or productivity [2]. In addition, studies by Weinberg *et al.* (2003) [33] and Hindrichsen *et al.* (2012) [34] have highlighted more favourable anaerobic ruminal fermentation conditions with silage *Lactobacillus* inoculants becoming part of the rumen microbe ecology so assisting in ruminal buffering and scavenging of oxygen. This idea of greater *Lactobacillus plantarum* has been supported by microbe DNA detection and fingerprinting with cows consuming inoculant-treated lucerne silage compared to non-inoculated [35]. However, an *in-vitro* study by Jalč *et al.* (2004) [36], using an artificial rumen technique (RUSITEC) to examine methane production between non-inoculated grass silage and inoculated with either *Lactobacillus fermentum* or *Enterococcus faecium* showed no difference in methane levels between treatments. Given the *in-vitro* nature of the study it does not account for what differences in DMI and productivity that might have occurred if it had been *in-vivo* and the possible dilution of methane over these parameters. This is an important aspect missed out by the authors especially when the *Lactobacillus* inoculated grass silage resulted in significant increase in material degradability ($P<0.05$) and levels of propionate ($P<0.001$), both factors known to assist in lifting DMI and therefore animal productivity.

Other Considerations

Exogenous fibrolytic enzymes (EFEs): Ruminal throughput is very much determined by the proportion of cell wall in the forage therefore influencing daily DMI and consequently ECH_4 production intensity. It follows if digestion of the forage cell wall can be speeded up and increased by breaking down the intricate bonds which exist between the components of the cell wall then ECH_4 can be mitigated [37,38]. The use of EFEs in doing this becomes an option and studies of their application have shown positive responses in altering the ensiled forage. Colombatto *et al.* (2004) [39] recorded a significant decrease in NDF and ADF ($P<0.05$) and an increase in organic matter degradability with *in vitro* studies when maize silage was treated with EFEs just before ensiling. Nevertheless, an *in-vivo* study, although showing significant positive changes in composites relating to digestability when EFEs were added to lucerne and barley whole crop showed no improvement in the performance of early lactation dairy cows [40]. Mendoza *et al.* (2014) [37] review considering EFEs picks up on these types of inconsistencies with other studies and highlights the cost of such products as being a huge deterrent in their adoption by farmers. However there appears to be a lack of quantifiable findings of use with EFEs on grass silages and their direct impact on methane production.

Ensiled tannin rich forage legumes: Tannin and saponins compounds are found in high concentrations in certain forages, such as sainfoin and have long been identified as reducing enteric methane largely because of their anti-microbial nature [41,10]. Supplementation of concentrated form of tannins although consistent in reducing methane has had serious effects on animals' DMI and significant losses in production. A study involving a low inclusion rate (163g.day^{-1} of condensed tannins to lactating dairy cows saw a 16% reduction in methane but worryingly a milk yield drop of 5% and milk solids of 8% [42]. Although these results are unviable, it did demonstrate real potential for ECH_4 reduction.

One study that has shown viability has been ensiled sainfoin; a legume rich in tannins. Huyen *et al.'s* (2016) [43] study of 50% exchange of sainfoin silage with grass silage to lactating dairy cows showed no changes in DMI before and after nevertheless milk yield increased significantly by 9.4% ($P=0.042$) and methane fell by 5.8% on a kg DMI basis, although not significantly. This study suggests this mitigation strategy is worth pursuing by the use of modern plant breeding technology. This may be in the form of plant genetics to breed tannin rich sainfoin varieties or other mainstream legumes and to overcome the low yields of sainfoin [44] making it more viable for commercial livestock units.

Conclusion

ECH_4 mitigation via ensiled forages is a welcomed strategy in the livestock industry not just for diminishing its impact as a greenhouse gas but also increasing productivity because of the energy contained in ECH_4 not being lost by the animal accompanied by an increase of ensiled forage intakes. Choice of forage type such as maize silage is an easy strategy to employ when ECH_4 mitigation is solely considered but needs to be questioned when full associated greenhouse gas production is taken into account. The role of harvesting early cut swards for ensiling is another strategy and needs greater prominence as a mitigation strategy. However, execution of best practise of ensiling and feed out is essential for it to be effective. The ensiling fermentation process can be assisted with *Lactobacillus* inoculants and can in their own right enhance the rumen's ecology and environment to assist in the mitigation of ECH_4.

Future considerations for ECH_4 mitigation could involve understanding how different strains of methanogenic *Archea* operate with different forages and exploring if there is anyway of manipulating them. Forages containing bioactive compounds also show promise and with the use of plant breeding may be a means of making this strategy practical at farm level. By making sure future strategies are functional on farm and highlighting the importance forage type, maturation and the precision of ensiling forages has on the reduction of ECH_4 then mitigating the impact of livestock on the environment can be attained.

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