A REVIEW: THE IMPORTANCE OF THE ENTERIC METHANE PRODUCTION MEASUREMENT METHODS AND MITIGATION STRATEGIES IN RUMINANT ANIMALS

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ABSTRACT

Ruminant animals are response to the emission of enteric methane due to the fermentation process which happens in their rumen and produced methane as a byproduct. Methane is one of the most important (GHGs) greenhouse gases because it is a major cause of global warming due to its ability to absorb heat in the atmosphere about 25 times more than carbon dioxide. An adult cow can belch about 400-500 liter methane/day, and this production causes a loss in the gross energy intake with a percentage from 2 to 12%. So, there are many strategies to mitigate the methane emissions from the ruminant animals that we will demonstrate in this work such as plant bioactive compounds, dietary lipids, exogenous enzymes, defaunation. On the other hand, there are many methods to measure the methane emissions from the ruminant animals such as the SF6 Tracer technique, open-circuit chambers, and headbox. The objective of this review is to evaluate options that have been demonstrated to mitigate enteric methane emissions produced by ruminants and the methane emission measurement options to be considered under Egyptian conditions.

***Keywords**: Enteric methane, ruminant animals, global warming, methane mitigation strategies, methane measurement*

INTRODUCTION

Enteric methane (CH4) emissions derived from the gastrointestinal tract of ruminants are approximately 10 times higher than the emissions from manure. Methane is one of the end-products of ruminal fermentation, formed autotrophically by methanogenic archaea from CO2 and H2 derived from the fermentation of carbon sources, in particular sugars (Demeyer and Fievez, 2000). Methane is finally eliminated by belching, representing a loss of between 5 and 8% of the gross energy contained in the feedstuffs consumed by the animal (Petherick, 2012). Enteric methane is also considered a major source of greenhouse gas emissions from agriculture (Moss *et al.*, 2000). Worldwide, approximately 81 Tg (tera-gram) (1 Tg = 1 million tons; Wahlen *et al.*, 1989) methane per year are emitted from the gastrointestinal tract of domestic ruminants and an additional 7 Tg of methane per year originate from the manure of these animals (Johnson *et al.*, 2000). Domestic ruminants are responsible for 25% of total anthropogenic methane emission (Johnson *et al.*, 2000).

1. Methane measurement methods

1.1. SF6 (sulfur hexafluoride) tracer technique, and sniffers

The SF6 method was described by Johnson *et al.* (2000) with specifications by Arbre *et al.* (2016). A permeation tube with a known SF6 gas release rate was introduced in the rumen of animals 1 month before the experiment. In general, 695.8 ± 59.9 mg of SF6 is introduced in the tube, and the permeation rate of SF6 from the tubes averaged 1.545 ± 0.055 mg/day. Lifetime of permeation tubes is 8.2 ± 1.7 months, i.e., enough to maintain a constant diffusion rate of the SF6 throughout the 15-wk experiment (Arbre *et al.*, 2016). Sampling is performed using a Teflon tube held close to the nostrils and a capillary tube connected to a cylindrical gas collection device (length: 37 cm; diameter: 9 cm; volume 2.5 L). Gas collection devices must be changed every morning before feeding. Gas analysis also was described by Arbre *et al.* (2016): 2 chromatographs were used, 1 with an electron capture detector for SF6, the other

with a thermal conductivity detector for CO2 and a flame ionization detector for CH4. The SF6 method could be used under the Egyptian condition, especially with the water buffalo, because it is an easy method to be applied on such animal difficult to deal with.

1.2. Open-circuit (OC) chambers

The OC measurement was described by Guyader et al., (2015). Each OC is 2.2 m high, 3.6 m long and 2.1 m wide, giving a volume of 16.6 m^3. Floor dimensions give the animal a 2 m^2 movement area, which is close to tie stall conditions and equipped with a comfortable rubber mattress. The chambers are made of steel with transparent polycarbonate walls allowing sight contact between animals and with the farm staffs. Chambers has front and rear doors, the front doors are used for animal feeding and the rear doors were used to enter or milk the animals, or to remove feces and urine collected once daily in a wheeled box. Chambers normally is calibrated the day before each measurement week using pure N2 and a mixture of CH4 (650 ppm) and CO2 (700 ppm) in N2. Airflow in the exhaust duct of each chamber is continuously measured (CP300 pressure transmitter; KIMO, Montpon-Ménestérol, France) and recorded every 5 min (KT-210-AO data logger; KIMO, Montpon-Ménestérol, France) (Guyader *et al.,* 2015). The OC system can be used on the dairy cattle and the sheep in Egypt.

1.3. Measuring methane and carbon dioxide emissions using Green Feed (GF)

The GF system has been developed by a private company (C-Lock Inc., Rapid City, SD, USA). It was described by *Arbre et al.* (2016). The GF device is an automatic feeder filled with a concentrate available for each animal up to 6 times a day with a 4-h interval. Each visit to the feeder allowed the intake of 300 g of concentrate in 6 successive 50-g drops. A device for air extraction allows the measurement of air outflow and of CH4 and CO2 concentrations in the extraction pipe by a non-dispersive infrared sensor. The calibration of the non-dispersive infra-red sensor of the GF is automatically performed twice a day by injecting gas mixture of certified concentrations of CH4 (1,003.4 ppm) and CO2 (9,997 ppm) in N2 (Air Liquide, Mitry-Mory, France). Methane and CO2 emissions (g/day) is normally calculated from CH4 and CO2 concentrations and air flow during the animal's visits to the feeder, corrected by background CH4 and CO2 concentration and air flow, and by air temperature. Data is normally transferred to the C-lock server in a blind manner and handled by C-lock. The GF method is high-cost method, but also can be used in Egypt to have a standardized result to be compared with the international results.

1.4. In vitro methane production measurements

This is a relatively cheap method that is acceptable for analyzing CH4 emissions from a huge variety of feed additives and plant extracts without the error of individual variation (Alvarez *et al.,* 2019). It is particularly useful for ranking different dietary interventions. The basic principle of every in vitro fermentation technique relies on the incubation of feed samples along with the rumen microbial inoculum and buffer solution in an anaerobic environment (Russo *et al.,* 2017). The anaerobic fermentation of feed samples can yield various gases in the container and the cumulative volume can be later recorded (Gonzalez-Rivas *et al.,* 2016). The typical gas compositions and CH4 concentrations can be estimated using the gas samples harvested from the headspace of the container (Russo *et al.,* 2017). The harvested gas samples are then evaluated using gas chromatography as explained by Gomaa *et al.* (2017), where the gas samples are moved into a vacuumed tube using a plastic syringe which is connected to a 3-way tap. The 3-way tape is helping to hold the gas samples closed in the syringe without losing it. The in vitro method is easy to be used in Egypt, due to its relatively cheap price.

2. Mitigation strategies of enteric methane production

Mitigation of enteric methane (CH4) emissions is a major challenge for the future of livestock farming due to its large contribution to greenhouse gas emissions.

2.1. The use of tannins to mitigate methane emissions

Many of the plant species have secondary compounds capable of changing the utilization of nutrients by mammalian herbivores. Because plants developed defense mechanisms against herbivores and pathogens, animals have developed mechanisms to nullify or restrict the toxic and negative effects of ingested plant secondary compounds such as condensed tannins (CT) (Coelho *et al.,* 2011).

In ruminants, Adejoro *et al.* (2020) showed that dietary supplementation with tannins (TANs) improved the utilization efficiency of ingested feed. In addition, TANs have been successfully used to reduce enteric CH4 production, urinary N excretion, and N2O emissions (Adejoro *et al.,* 2020; Fagundes *et al.,* 2020) and to increase the duodenal flux of microbial protein and amino acids (Orlandi *et al.,* 2015). TANs-rich plants and TANs' extracts have also shown positive impact on rumen microbial

activity (Sarnataro and Spanghero, 2020), ruminal fermentation rate (Fagundes *et al.*, 2020), antioxidant status, and health of ruminants (Santos et al., 2021). TANs can also reduce the digestion of protein in the rumen and the entire gastrointestinal tract (Waghorn, 2008). Therefore, the intake of TANs in combination with a medium-poor quality diet (e.g., insufficient crude protein in the diet) may not generate nutritional benefits and is detrimental to performance (Seoni *et al.*, 2021). For example, some studies have reported negative effects of dietary supplementation with TANs on digestibility, productive performance, and ruminal fermentation (Adejoro *et al.*, 2020), while other studies have not observed significant effects on digestibility, productive performance, CH4 emissions, and urinary and fecal nitrogen excretion in response to TAN supplementation (Adejoro *et al.*, 2020).

Ruminant herbivores and plant CT coexist and adapt natural evolutionary processes. Some ruminant feeders, especially goats, developed physiological adaptations, and even dependence on CT-rich legumes, selectively including such plants in their selector habits (Muir *et al.*, 2011). The evolution of different feeding strategies among domestic ruminant species implies differing microbial interactions with CT and, consequently, the diversity of rumen microorganisms and digestive capacity.

The tannin content of the most Egyptian plants has not been evaluated yet. Although it is a good alternative method to mitigate enteric methane production.

2.2. The use of dietary lipids to mitigate methane emissions

The use of lipid compounds offers another possible strategy to decrease enteric CH4 emission from ruminants. Addition of lipid compounds inhibits the methanogenic and ciliate protozoan population in the rumen (Grainger and Beauchemin, 2011). Lipid addition also decreases organic matter and fiber degradability and reduces fermentable substrate to reduce CH4 production (Knapp *et al.*, 2014). Machmüller and Kreuzer, (1999) suggested coconut oil as an efficient natural additive to reduce CH4 production without causing detrimental effects on the nutrient utilization of the animals. On average, they observed 28 and 73% reductions in daily CH4 emission/animal when the Swiss Brown were housed in respiratory chambers are fed with a ration containing 3.5 and 7% coconut oil, respectively. The reduction in CH4 release could be due to the suppressive effect of coconut oil on methanogens and ciliate protozoa populations. Using soybean oil, Mao *et al.* (2010) demonstrated around a 13.9% decrease in CH4 production in Huzhou lambs when measured using a simple, open-circuit respiratory chamber. Similarly, Chuntrakort *et al.* (2014) investigated the effect of different feeding oil plant diets on CH4 emission using a headbox respiration chamber system from Thai native Brahman crossbred cattle and observed a reduction in CH4 production with oil supplementation.

2.3. The use of exogenous enzymes to mitigate methane emissions

Exogenous enzymes are widely used to remove the anti-nutritional factors in livestock feed and to improve digestibility (McAllister *et al.*, 2001). The enzymes are generally sourced from bacteria such as Lactobacillus acidophilus, Streptococcus faecium, spp., and Bacillus subtilis, and fungi like Trichoderma reesei, Aspergillus oryzae, and Saccharomyces cerevisiae. Spp. The studies linking CH4 production and exogenous enzymes are very limited and confusing. Arriola *et al.* (2011) tested the effect of a fibrolytic enzyme on CH4 production from two groups of Holstein cows fed low- and high-concentrate diets, respectively, and they observed a reduction in CH4 production when the animals were supplemented with the fibrolytic enzyme; these animals were housed in a free stall, open-sided barn. Further, the effects were more prominent in the high-concentrate-based diet. Zhao *et al.* (2015) demonstrated a reduction in CH4 production from feed substrates supplemented with cellulose and xylanase enzymes and tested in vitro.

2.4. The use of defaunation to mitigate methane emissions

Rumen protozoa are important, but not essential in the rumen ecosystem and to the well-being of host animals (Newbold *et al.*, 2015). Removal of rumen ciliate protozoa (defaunation) increased growth rate and live weight gain of ruminants (Newbold *et al.*, 2015) especially when the feed is deficient in protein relative to energy content. In addition, rumen protozoa are significant hydrogen (H2) producers and synthesis mainly acetate and butyrate rather than propionate (Williams and Coleman, 1992). Rumen ciliates are not observed in newborn animals, but they are passed from mother to offspring by direct transfer of saliva containing the active protozoa (Stewart *et al.*, 1988). Therefore, rumen ciliate protozoa are not present in animals at birth, enabling protozoa-free animals to be established by separating offspring from their mothers (Ivan *et al.*, 1986).

Capric acid (C10:0), lauric acid (C12:0) and rnyristic acid (C14:0) show strong protozoal toxicity and are useful as rumen defaunation agents (Matsumoto et al., 1991). Matsumoto *et al.* (1991) observed that rumen protozoa, except Entodiniurn spp., were undetectable after 3 days of feeding 30 g of hydrated

coconut oil (CO) containing 52% lauric acid. Feeding 250 g of refined CO to beef heifers reduced rumen protozoal population by 62% (Jordan *et al.,* 2006).

However, the percentage emission reduction was variable and mechanisms by which CH4 emissions are reduced by defaunation are not clear. Hegarty (1999) proposed four possible mechanisms by which defaunation induces a lower CH4 emissions, being: (1) reduced DM fermentation in the rumen, (2) decreased endosymbiotic methanogens associated with rumen protozoa, (3) modified ruminal VFA profile with increased molar proportion of propionate and decreased availability of H2, and (4) increased oxygen pressure in rumen fluid.

In addition, as CH4 production is not always decreased by defaunation (Kumar *et al.,* 2013), alternative methanogen populations may arise and replace those of the protozoa-associated methanogens (Morgavi *et al.,* 2012). The changes in the methanogenic community following defaunation are inconsistent among studies (Morgavi *et al.,* 2012; Kumar *et al.,* 2013).

2.5. The use of 3-nitrooxypropanol to reduce methane production

Several dietary strategies have been proposed to mitigate enteric CH4 production, including the use of feed additives. Some feed additives are inhibitors of methanogenesis, natural or synthetic compounds that directly inhibit methanogenesis by rumen archaea. Recently, Duin *et al.* (2016) described the characteristics of the feed additive 3-nitrooxypropanol (3-NOP). The compound 3-NOP is a highly specific inhibitor that targets the nickel enzyme methyl-coenzyme M reductase, which catalyzes the final step in methanogenesis in rumen archaea (Duin *et al.,* 2016). At low concentrations, 3-NOP appears to inhibit methanogens without having a negative effect on performance in dairy cattle (Hristov *et al.,* 2018). Melgar *et al.* (2020) investigated the effect of 3-NOP fed to dairy cattle on a corn silage-based diet throughout the entire early lactation period, starting from onset of lactation until 105 days in milk (DIM). In that study, emissions of both CH4 and H2 were measured with the Green Feed system. Gastelen *et al.* (2020) found that feeding 3-NOP did not affect dry matter intake (DMI), milk yield, milk component yield, or feed efficiency. The 3-NOP is an effective strategy to decrease CH4 emissions (while increasing H2 emission) in early lactation Holstein-Friesian cows with positive effects on apparent total-tract digestibility of nutrients.

CONCLUSION

It can be concluded that some of the methods of methane measurements are suitable to be used under the Egyptian condition due to the cost of the method, and the animal species, and due to the Egyptian desert weather conditions. Methods such as SF6, greenfeed, and Open-circuit chambers could be used. There are many unstudied plant species on the rumen enteric methane mitigation, also the absent of some new chemicals such as 3-Nitrooxypropanol in our laboratories.

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بحث مرجعى: أهمية طرق قياس إنتاج الميثان الداخلى وإستيراتيجية الحد منه فى الحيوانات المجترة

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الملخص العربى

الحيوانات المجترة هي المسؤلة عن انبعاث غاز الميثان المعوي بسبب عملية التخمر التي تحدث في الكرش ويتكون الميثان كمنتج ثانوي. الميثان هو أحد أهم الغازات الدفيئة ، لأنه سبب رئيسي للاحتباس الحراري العالمي بسبب قدرته على امتصاص الحرارة في الغلاف الجوي إلي حوالي 25 مرة أكثر من ثاني أكسيد الكربون. ويمكن للبقرة البالغة أن تنتجشأ حوالي 400-500 لتر / يوم من غاز الميثان، وهذا الإنتاج يسبب خسارة في إجمالي استهلاك الطاقة العلفية بنسبة 2 إلى 12٪.

هناك العديد من الاستراتيجيات للتخفيف من انبعاثات الميثان من الحيوانات المجترة التي سنعرضها في هذا العمل مثل المركبات النشطة بيولوجيًا في النبات ، والدهون الغذائية ، والإنزيمات الخارجية ، والتخلص من البروتوزوا.

من ناحية أخرى ، هناك العديد من الطرق لقياس انبعاثات الميثان من الحيوانات المجترة مثل تقنية SF6 Tracer سداسي فلورو الكبريت ، وطريقة الغرف التنفسية ذات الدائرة المفتوحة . الهدف من هذه المراجعة هو تقييم الخيارات التي تم توضيحها للتخفيف من انبعاثات غاز الميثان المعوي التي تنتجها المجترات وخيارات قياس انبعاثات الميثان التي يجب مراعاتها في ظل الظروف المصرية.

الكلمات المفتاحية: *الميثان المعوي ، الحيوانات المجترة ، الاحتباس الحراري ، استراتيجيات تخفيف غاز الميثان ، قياس الميثان*