



## Review Article

## Equine Contribution in Methane Emission and Its Mitigation Strategies

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## ABSTRACT

Greenhouses gas emission mitigation is a very important aspect of earth sustainability with greenhouse gasses reduction, a focus of agricultural and petrochemical industries. Methane is produced in nonruminant herbivores such as horses because they undergo hindgut fermentation. Although equine produce less methane than ruminant, increasing population of horses might increase their contribution to the present 1.2 to 1.7 Tg, estimate. Diet, feeding frequency, season, genome, and protozoa population influence methane production equine. In population, *Methanomicrobiales*, *Methanosarcinales*, *Methanobacteriales*, and *Methanoplasmatales* are the clade identified in equine. *Methanocorpusculum labreanum* is common among hindgut fermenters like horses and termite. Naturally, acetogenesis and interrelationship between the host and the immune-anatomical interaction are responsible for the reduced methane output in horses. However, to reduce methane output in equine, and increase energy derived from feed intake, the use of biochar, increase in acetogens, inclusion of fibre enzymes and plant extract, and recycling of fecal energy through anaerobic gas fermentation. These might be feasible ways to reducing methane contribution from horse and could be applied to ruminants too.

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## 1. Introduction

Greenhouse gases emission mitigation is an important aspect of earth sustainability. There has been a shift in research focus from methane (CH<sub>4</sub>) being an energy loss to CH<sub>4</sub> in herbivores as greenhouse gas because of its contribution to global warming processes [1]. Both agricultural and petrochemical industries are committed to reducing greenhouses gas emission with CH<sub>4</sub> being a focus of both “agrochemical–petrochemical” industry. Canadian oil and gas sector has committed to reducing CH<sub>4</sub> emission in 2025 by 40% to 45% from the volume recorded in 2012 [2].

*Animal welfare/ethical statement:* The research was performed in accordance with the ethical standard laid down in the 1996 declaration of Helsinki and its later amendments.

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Agriculture contributes to greenhouse gasses through livestock production and agronomic practices. Of these practices, monogastric (herbivores and omnivores) and ruminant contribute largely to this. In agronomy, marshes, swamp area, and rice paddies contribute their quota to global greenhouses gas concentration. CH<sub>4</sub> emissions are linked with its disadvantage on the environment (global warming effect) and its negativity as energy loss processes in foregut and hindgut fermenter where it is produced, thereby reducing beneficial energy that could be derived/personally useful to the animal for repair, maintenance, and growth.

Herbivorous animals, such as horses, donkeys, mules, and hinnies, consume mainly fibrous feeds and emit higher amount of CH<sub>4</sub> than other monogastric that eats nonfibrous diet [3–5], as a result of microbial fermentation processes in their hindgut [6], which allows methanogens that uses CO<sub>2</sub> alongside hydrogen (H<sub>2</sub>) for methanogenesis [7]. It is estimated that with the population of world horses at 58.8 million [8], CH<sub>4</sub> emission is expected to be about 1.1 Mt per year [3], which represents 26.5 Mt CO<sub>2</sub> equivalent per year, and around 0.6% of the global greenhouses gas emissions from cattle [9]. Horses produce 3.3 times less (92 ± 15 vs. 28 ± 9 L/

kg) CH<sub>4</sub> than ruminant per unit of digested neutral detergent fiber [10]. CH<sub>4</sub> production increases with increasing content of fiber in feed [11]; and because horses consume pasture mainly, it could be assumed that with increasing population of horses in the world, CH<sub>4</sub> from equine might be increasing. Among herbivorous monogastric, CH<sub>4</sub> is produced in large quantity in animals such as mules, horses, and asses because they are hindgut fermenters [12].

Presently, there is scarcity of information about the CH<sub>4</sub> population and mitigation strategies in horses and hindgut fermenters, and most knowledge of the microbial population of horses have been derived from the fecal analysis. Thus, this review is to give a brief on literary evidence of methanogens in the gut and proffers few mitigation strategies.

## 2. Horses/Digestive System

Horses are neither ruminant nor are they pure monogastric. Although they are herbivores, they share some digestive similarities with both classes of animals such as stomach, small intestine, and large intestines. They undergo fermentation in their modified caecum–colon chamber similar to the rumen in function. The caecum–colon of equine is a fermentation vat where microbial breakdown and nutrient and fluid absorption from digesta that have undergone enzymatic partial digestion take place. In this chamber, short-chain fatty acid and other gasses are obligatorily produced at varying rate depending on the diet consumed (Fig. 1).

## 3. Production of Less CH<sub>4</sub> in Horses

There are contrasting microbial community structure/profile in the digestive tract between the ruminant and equine [10] and possibly among methanogens, which results in the production of CH<sub>4</sub> at varying quantities even when they are placed on the same standard such as body weight or feed intake. The reason for such difference in CH<sub>4</sub> output as reported by Franz et al [10] was attributed to shorter retention time for holding food in equine or increase in *Archaea* community. Furthermore, Leng [14] attributed this methane output differences, to the structural variation that

exist in the design/anatomy of each species fermentative organ and the location of these organs in the gastrointestinal tract in structure (the rumen in ruminant and cecum–colon in horses) while in position (rumen in the foregut and caecum–colon in the hindgut). In addition, it is reported that protozoa and archaea are lower in equine compared with ruminant [15,16]. The implication of this is that such microbial population variation in equine would lead to reduction in the symbiotic H<sub>2</sub> exchange relationship existing between protozoa and methanogen. Hence, the reduced CH<sub>4</sub> emission in horses. Perhaps, there is an alternative H<sub>2</sub> sink aside methanogenesis in the hindgut. Franz et al [10] and Leng [14] suggested that their acetogenesis seem to be other alternative H<sub>2</sub> sink, which might be peculiar to hindgut feed degrader.

## 4. Hindgut Fermenters

Rodents, rabbits, koalas, horses, rhinoceros, elephant, and termites are examples of hindgut fermenters. Efficient digestion of fiber and roughages in equine and other hindgut fermenters are made possible in a similar pattern as in the rumen by the presence of microbes in the hindgut which aid fermentation [17]. Hindgut fermenters rely on the microbial communities for the majority of their energy in the form of volatile fatty acids. Hindgut microbes are very important to the health and digestibility of horses. Disruption of the balance in the hindgut microbial community by factors, such as diet and antibiotics, causes digestive disorder, acidosis, and pathogenic increase. Therefore, balance in gut microbes is essential for maintaining gut wall integrity and contribute to preventing disorders and formation of blockade against disease-causing microbes [17].

Efficient digestion in the hindgut (caecum–colon) of horses may be attributed to the dense biofilm of microbes, which allow complex interaction between microbes and feed surface to provide protein and energy requirement for large monogastric herbivores [14]. Although fermentation occurs in the caecum–colon chamber, there are variations in the microbial population in the colon and caecum chamber, such that there are higher population of fibrolytic bacteria in the caecum, whereas amylolytic bacteria (lactic acid

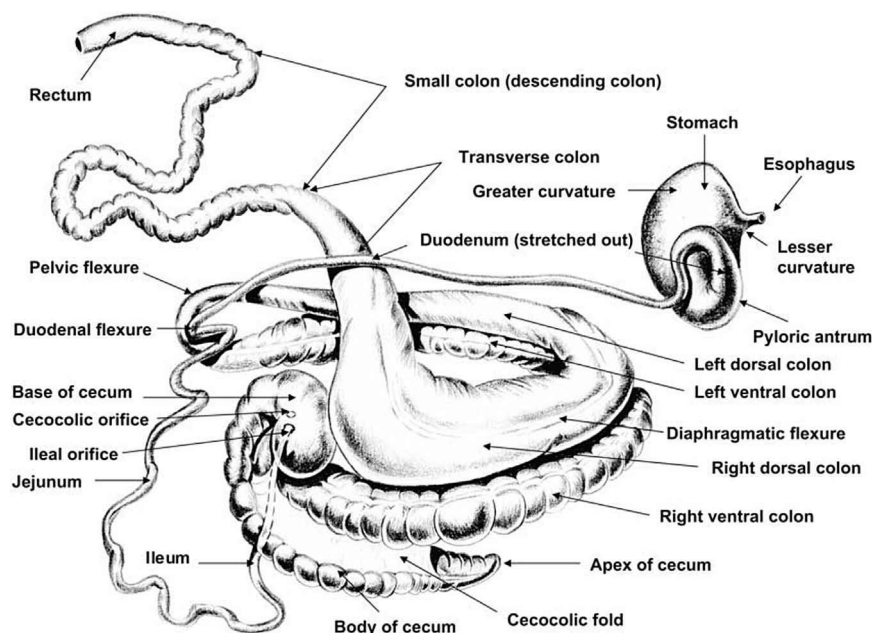


Fig. 1. Equine digestive system [13].

producer, lactic acid utilizers, and *Streptococci*) are higher in the colon [15,18–20].

## 5. Equine Gut Microbes and Factors Influencing Them

Quick transformation of the microbiome occurs in the gut of neonate from birth till day 56 and in adult horses which is most likely caused by several factors mentioned below but chiefly diet [21]. The microbes existing in the gut are bacteria, fungi, protozoa, archaea, and viruses that are capable of degrading and fermenting structural polysaccharides of the plant cell walls [22], and they exhibit close between-species and species–host tissue associations [14]. These microbes are phylogenetically diverse groups, which function mutually dependent through complex trophic relationships [23]. Large portion of equine gut microbiota is unique to individual animal, and microbes in different gastrointestinal tract compartment are very different from one another in richness [24]. The diet of animal contains carbohydrate, protein, cellulose, fiber, hemicellulose, and starch of varying rate of degradation, and they yield different products after fermentation. Diverse microbes exist in the gastrointestinal tract of ruminants (cattle sheep and goat), nonruminant (pig, poultry), and hindgut fermenters (horses, rabbit). They contribute to digestion, animal health, and ethics/welfare. The microbial diversity in these organisms is borne out of microbe's preference for different substrates and resulting in the production of different metabolites such as microbial protein, acetate, propionate, butyrate, CH<sub>4</sub>, and other iso-acids. Furthermore, the preference of microbes to different endo-environmental conditions, especially gut pH, contributes to this. As a result, the diversity of microbes in the gut at times will be determined by the varieties of substrate accessible in the diet.

Despite the acidity in the stomach of horses, 10<sup>6</sup> to 10<sup>8</sup> cfu/mL of microbes are found in the stomach. Julliand [25] isolated *Lactobacillus salivarius*, *Lactobacillus crispatus*, *Lactobacillus reuteri*, and *Lactobacillus agilis* from the nonsecreting region of the stomach. Similarly, *Lactobacillus mucosae* and *Lactobacillus delbrueckii* were isolated in the stomach of forage fed horse [26] while [27] identified lactate-utilizing bacteria, *Streptococci*, and *Lactobacilli*. This indicates that *Lactobacilli* is probably the dominant microbe in the stomach, which is very plausible because of the penchant of *Lactobacilli* to low acidity.

In the small intestine, 10<sup>6</sup> to 10<sup>9</sup> cfu/mL of strict anaerobic gut microbes, which include *Lactobacilli*, *Enterobacteria*, *Enterococci*, *Streptococci* and lactate-utilizing bacteria, novel *Lactobacilli ruminis* [28], and *Streptococci*, are dominant [21]. Overall, there are higher levels of total anaerobic bacteria in the small intestine and stomach than in the hindgut of those fed concentrate diet, whereas there is homogeneity of microbes in the gastrointestinal tract of those feeding on forages [17]. This could be because of the disparity of pH, which is influenced by the quick degradability of the substrate in each diet and the feed that escaped proper digestion in the stomach, which would go to the hindgut, thereby creating a more acidic environment, which is in contrast to fiber diet, which tend to increase the pH of the gut during microbial fermentation. Because of almost neutral pH and slow passage rate in the large intestine, there is increased diversity and higher number of microbes in the cecum and colon at about 10<sup>9</sup> and 10<sup>8</sup>, respectively [29]. Caeca fungi are about 10<sup>1</sup> to 10<sup>4</sup> per milliliter [25], whereas protozoa content of caecum and colon is between 10<sup>3</sup> to 10<sup>5</sup> per milliliter with variation in individual animals [15,26]. Higher number of cellulolytic microbes are found in the caecum compared with the colon, which is an indication of more fiber fermentation in the caecum, slower passage rate, and favorable pH [30]. *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* are the main cellulolytic bacteria in the caecum of horses [17] with *Eubacterium*

spp., *Ruminococcus* spp., *Clostridium* spp., and *Butyrivibrio* spp. being the most important cellulolytic microbes which are similar to ruminant [7].

Several ruminal and hindgut bacterial genera can ferment lactic acid including *Anaerovibrio*, *Megasphaera*, *Fusobacterium*, *Peptostreptococcus*, *Selenomonas*, *Propionibacterium*, and *Veillonella* and *Megasphaera elsdenii* are believed to be the most important [31]. In the caecum–colon, *L. mucosae*, *L. delbrueckii*, *L. salivarius*, *Mitsuo-kella jalaludinii*, *Streptococcus bovis*, and *S. equinus* have been identified as the dominant lactic acid producer, whereas *Veillonella* sp. and *Megashpera* sp. are the primary lactate-utilizing inhabitants of the caecum–colon [25].

Costa et al [32] observed that there are variations in the microbes in different sector of the horse intestine vary. *Bacilli* were predominant in the stomach and duodenum with more than 50 and 80%, respectively, and <2% in the cecum at the class level. At phylum level, *Firmicutes* was dominant in all the gut sector in a range of 85 to 95% in the stomach and duodenum; this is because *Firmicutes* has a preference for grain/starch diet, which is usually common in the diet of modern-day horses. *Sarnia*, *Lactobacillus*, and *Streptococcus* are the foremost genera of *Firmicutes*. Within the *Firmicutes*, *Clostridia* dominated microbes in the hindgut (cecum, pelvic flexure, small colon, rectum, and feces) with more than 80% present in them, and *Bacilli* dominated the duodenum, stomach, and ileum at more than 80, 50, and 30%, respectively. Toward the hindgut, that is, cecum < pelvic flexure < small colon < rectum < feces, there was an increased level of *Fibrobacteres* in contrast to the stomach and duodenum. *Protobacteria* population was the highest in the ileum of the horses.

Most knowledge obtained of equine intestinal microbial population or classification are obtained from the fecal sample because it is noninvasive. It is estimated that 30–80% of microbes in the cecum–colon are strictly anaerobic [33,15]. Total anaerobes ranges between 1.85 × 10<sup>7</sup> and 2.65 × 10<sup>9</sup> cfu/mL in the caecum [34]. As observed by Kobayashi et al [35], microbial population of bacteria (gram-negative rod and cocci) and protozoa seem to vary with season. For instance, in both light horses and native horses, bacteria population in the summer was 3.7-fold higher than the winter in native horses and 6.6-fold higher in light horses in the summer than the winter. This also affects the population of the microbes as gram-positive and gram-negative rod microbes were higher than cocci, while gram-negative microbes were higher than gram-positive in the feces. Total number of protozoa was also affected by season with high number occurring in the summer than in the winter. Individually, *Bundleia postciliata* (39.2–54.7%), *Ditoxum funinucleum* (2.0–15.8%), *Triadinium caudatum* (0.1–8.3%), *Tri-palmaria dogieri* (4.8–16.4%), *Didesmis quadrata* (5.8–17.2%), *Cycloposuhium bipalmatum* (3.2–12.2%), *Cochliatoxum periachtum* (2.1–5.9%), and *Blepharocorys uncinata* (4.3–23.2%) were the protozoa detected in the feces. The variation in microbial population in season (in both bacteria and protozoa) might be attributed to the variation in quality of grasses in both seasons. In the winter, the fibrous content of the forages would have been higher, thereby reducing digestibility and ease of access to nutrient. In addition, this could be seen in the population of protozoa across both seasons. Protozoa tend to increase when there is availability of soluble sugar or starch granules. For fiber degradation, protozoa seem to play some roles in it; as Moore and Dehority [26] noted that small protozoa seem to play little role, while large protozoa seem to plant important role in fiber degradation [35].

Breed of animals also seems to cause variation in the dominance of individual microbes even when they are from the same class. For instance, Julliand et al [36] pointed out that *R. flavefaciens* was of high importance in fiber degradation in horses. Contrariwise, Lin and Stahl [37] pointed out that in Hokkaido native horses,

*R. flavafaciens* is a minor species in the hindgut, whereas *F. succinogenes* was dominant/crucial for fiber digestion in horse hindgut. In the study of Yang [38], acetogens in hindgut fermenters (horses and rabbit) were more diverse than in foregut fermenter (cow and sheep) and indication of individual animal influence on its gut microbial diversity.

In horses, the cecal microbiota is influenced by frequency of feeding, and the when the frequency of feeding is higher, it is related to the increase in genus YRC22 and reduction in the population of *Prevotella*, *Streptococcus*, *Lactobacillus*, and *Coprococcus* [39,40].

## 6. Methanogens in Horses

Literary reports on methanogen population in equine are limited. *Archaea* act as H<sub>2</sub> sinks, converting H<sub>2</sub> and CO<sub>2</sub> to CH<sub>4</sub>, to keep the partial pressure of H<sub>2</sub> low [10]; this process enhances the activity of fermenting microorganisms in the gut ecosystems [12]. The existence of methanogens in the gut of vertebrates and invertebrates has been reported by Yuki et al [23]. It is known that methanogens have the ability to reduce CO<sub>2</sub> to CH<sub>4</sub> by converting several substrates such as H<sub>2</sub>, formate, acetate, methanol, and methylamines during fermentation [41].

All presently known methanogens belong to the phylum *Euryarchaeota* and the order *Methanosarcinales*, *Methanomicrobiales*, *Methanococcales*, *Methanocellales*, *Methanobacteriales*, *Methanomassiliicoccales*, and *Methanopyrales* [42]. Knowledge of the diversity of methanogens in the gastrointestinal tract of horse is also important for understanding the mitigation of CH<sub>4</sub> emission [43].

Luo et al [44] reported that in feces of white rhinoceroses, *Ceratotherium simum*, *Methanocorpusculum labreanum*, *Methanobrevibacter smithii*, *Methanosphaera stadtmanae*, and *Methanomassiliicoccus luminyensis* were identified and occur sequentially at 42, 27, 4, and 2%, respectively, of the total sequences analyzed. The phylogenetic analysis of hindgut methanogens in white rhinoceroses is more comparable to that of horses [44]. *M. labreanum* is also found in the hindgut of termites. In addition, methanogens population also varies in population in different anatomical section of the colon. However, *M. labreanum* was common in all the hindgut section (*Methanobrevibacter ruminantium* and relatives and *Methanocorpusculum* and relatives) in equine feces [45].

In the study of Zhang et al [46] where horse manure was codigested with food waste, 12 archaea genus were identified, and only *Methanosarcina*, *Methanobacterium*, *Methanosaeta*, and *Thermogymnomonas* were dominating, and they account for more 92% of the overall archaea population. Although *Thermogymnomonas* belongs to archaea in the order of *Thermoplasmatales*, it cannot produce CH<sub>4</sub> because it is not a methanogen [46]. However, *Methanosarcina* has the ability to produce CH<sub>4</sub> using different substrates such as acetate, H<sub>2</sub>/CO<sub>2</sub>, and methylated one-carbon compounds [41]. Thus, in vivo presence of *Methanosarcina* will most likely increases methanogenesis.

In the study of Lwin and Matsui [43], Clades—*Methanobacteriales*, *Methanomicrobiales*, and *Methanoplasmatales* clade—have been identified in horses and pony feces. Phylogenetic analysis showed that methanogens in horses are classified into four clades—*Methanomicrobiales*, *Methanosarcinales*, *Methanobacteriales*, and *Methanoplasmatales*, with *Methanomicrobiales* being the highest. In addition, *Methanobrevibacter gottschalkii* are available, in genus *Methanobrevibacter*, and *Methanomicrobiales* show high level in horses and pony. However, there are suggestions that rectal samples are not full representation of microbes in the small and

large intestines [22] of horses. Still, it gives us insight into the methanogen population in the horses.

## 7. CH<sub>4</sub> Production in Horses/Hindgut Fermenters

Mandatory H<sub>2</sub> production during feed fermentation is captured in the form of CH<sub>4</sub> and removed by eructation in ruminant or through acetate in the macropod foregut and equid caecum—colon [14].

Fermentation in the hindgut and foregut or ruminant and nonruminant herbivores, such as equine and cattle, sheep, and goat, lead to the production of similar metabolites such as volatile fatty acids, CH<sub>4</sub>, and other potent and less potent gasses with varying level of concentration and at different rates. Some of these variations are because of structural differences in the gut, microbial profile, and alternative sink for the gasses (methanogenesis and acetogenesis) and so on. Certainly, H<sub>2</sub> sinking is healthy for gut fermenters because it helps to maintain the fermentative processes, as hindrance to the H<sub>2</sub> gas reduction would increase the partial pressure in the gut and such could affect other respiratory, circulatory, excretory, and metabolic organs in the visceral [14].

Recent knowledge revealed that host (individual animal) is involved in determining the gut microbial consortium and the fermentation byproduct including gasses produced in the foregut of ruminant and the hindgut of horses [14]. The CH<sub>4</sub> emission in foregut and hindgut fermenter is brought about by the welfare practice of reducing the H<sub>2</sub> pressure in the fermentative vat to ensure survival through continuous fermentation of these animals. However, the internal gas homeostasis of these livestock is detrimental to the environment. There are many research articles and reviews on mitigation strategies, which are focused on ruminant because of their main contribution to the mild (CH<sub>4</sub>) and harsh (N<sub>2</sub>O), which has 25- and 298-folds damaging potency than CO<sub>2</sub> [47].

Methanogens and other hydrogenotrophs practice methanogenesis by positioning themselves on the outer layers of the biofilm where they access the H<sub>2</sub> diffusing from the site of carbohydrate fermentation [48] and then combine it with CO<sub>2</sub> to produce CH<sub>4</sub>. Johnson and Ward [49] estimate that CH<sub>4</sub> produced in the hindgut of pigs and horses to be about 0.9 to 1.0 Tg and 1.2 to 1.7 Tg, respectively. Jensen [12] reported that *Methanobrevibacterium* is the dominant methanogen in horse, which is also common in foregut of ruminant. This indicates that horses also produce CH<sub>4</sub>. The amount of CH<sub>4</sub> produced depends on the amount of nonstarch polysaccharide intake.

CH<sub>4</sub> production in monogastric that consume herbivores such as horse and mule is up to 80 L/d [12]. Methanogens are present in rat, pig, monkey, rhinoceros, hippopotamus, giant panda, poultry goose, and turkey (*Methanogenium*). CH<sub>4</sub> is produced from two *in vivo* anaerobic systems, which are positional different; in ruminant, it is produced from the rumen, while it is produced from the large intestine of monogastric from the caecum—colon.

There is a correlation between CH<sub>4</sub> output and concentration of fiber in the diet [50,51], especially when the fiber is not easily digestible, for example, acid detergent fiber, which should encourage the use of readily digestible ingredient which is common among carbohydrate. However, digestive problems are caused by the use of concentrate and high grain in the diet of horses which is fed to provide nutrient required to meet modern-day performance. However, the use of fibrous diet has been recommended to be included in the diet to combat digestive problems such as hindgut acidosis, colic, laminitis, and so on. Therefore, CH<sub>4</sub> emission in equine should be observed too. Although CH<sub>4</sub> emission per equine is small, any country that wants to delve into full horse production or where horses are more than the large or small ruminant, then

horses might be a chief contributor to livestock CH<sub>4</sub> in such country. CH<sub>4</sub> mitigation in horses could be *in vivo* or in an anaerobic environment (outside the body; Fig. 2).

## 8. Mitigation Strategies

CH<sub>4</sub> production in equine represents about 1.5 and 3.2 of gross energy and digestible energy, respectively [10], from the diet consumed by equids. Yet, energy losses per unit of body mass in rabbit and horses are significantly lower in comparison to ruminant [53] because of CH<sub>4</sub> production process. Sequel, to the volume of CH<sub>4</sub> produced by anthropogenic activity and nature itself, the influence of CH<sub>4</sub> on the globe, could have been worse had nature not devised means of degrading/removing CH<sub>4</sub>. Broucek [54] reported that CH<sub>4</sub> sunk each year is estimated to be 576 Mt per year out of the total 598 Mt produced annually, and stratospheric oxidation consumes 40 Mt per year, whereas about 30 Mt CH<sub>4</sub> are removed yearly from the atmosphere by soil uptake. This shows that 96.3% of CH<sub>4</sub> produced annually are sunk, with stratospheric oxidation removing 6.9% of total sunk and the soil taking up 75% of atmospheric CH<sub>4</sub> (calculated based on stratosphere consumption). Therefore, only 22 Mt are not consumed per year. This indicates that there is a need to find ways to reduce CH<sub>4</sub> output. Although, the CH<sub>4</sub> output from monogastric and hindgut fermenters is not much of a concern compared with cattle. Still, reducing CH<sub>4</sub> output from them would contribute to CH<sub>4</sub> reduction although little, it could go a long way.

Dietary manipulation, use of plant extract, yeast, plant seed, plant secondary metabolite, organic acids, and chemical (nitrate and sulfate), which subsequently result in alteration in rumen fermentation characteristics, are methods of reducing CH<sub>4</sub> production *in vivo* and *in vitro*. However, dietary manipulation has been suggested to be the one of the most potent ways of doing this [55]. Naturally, CH<sub>4</sub> emission is mitigated hypothetically, on relationship between internal anatomy and the responses of the host-tissue, that modifies the species of microbes in the intestine [14].

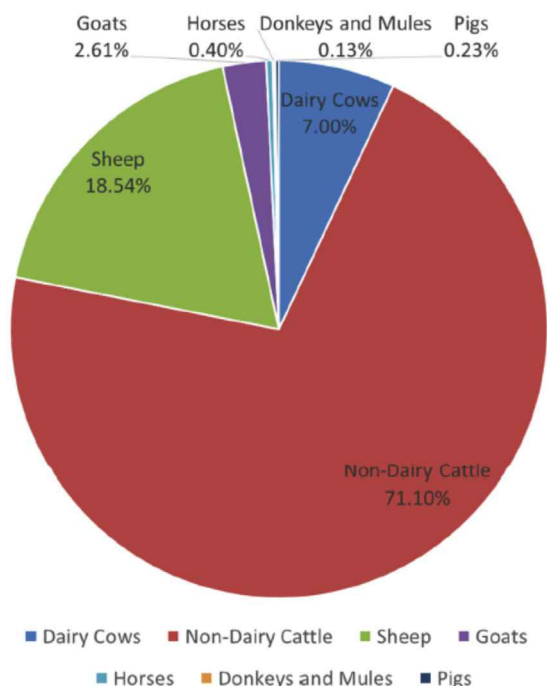


Fig. 2. Methane emission (kg CH<sub>4</sub> animal<sup>-1</sup> y) from livestock [52].

These host tissues release immune secretions, such as immunoglobulin and instinctive lymph cells that remove or overwhelm CH<sub>4</sub>-producing *Archaea*, and enhance the proliferation of acetogens [33] (Table 1).

### 8.1. Acetogenesis

Any CH<sub>4</sub> mitigation stratagem that is focused on reducing methanogenesis must provide an alternative route for H<sub>2</sub> sinking [60], as failure to do so would increase H<sub>2</sub> pressure, fermentation processes, and potential organ damage. Acetogenesis seem to be the natural way by which hindgut fermenters sink H<sub>2</sub>. In ruminant, inhibition of methanogenesis could enhance acetate concentration in the ruminal fluid with about 13 to 15% energy as benefit [61], and supposedly so in hindgut, fermenters such as equine, insect, and so on, for instance, *Blautia* spp. play important roles in kangaroo, which produces little CH<sub>4</sub> with increased acetogenesis as a major H<sub>2</sub> sink [62] and [38] observed that the dominant acetogens in the feces of horses and the ceca of rabbits were both affiliated with the *Blautia* group, which can produce acetate with H<sub>2</sub> and CO<sub>2</sub> or CO [63] with a least doubling time of 5 or 1.5 hours, respectively [64].

There are acetogen strains that grow using H<sub>2</sub> and organic substrates simultaneously and such would have energy and competitive advantage than methanogens [65]. Methanogenesis seem to be the most efficient H<sub>2</sub> sink in the rumen, whereas acetogenesis is the one in hindgut fermenter that does not possess eructation and flatulence frequency like ruminant although gasses are produced during their fermentation.

In Yang [38], acetogens enrichment from horse feces produced higher amounts of butyrate. Butyrate is an epithelial fuel, which favors its proliferation and intestinal health. However, the hydrogen reduction potential of acetogens or hydrogen used by horse acetogens during acetogenesis for one mole of acetate is 4.75 compared with cow, sheep, rabbit 5.53, 5.23, 5.17 moles respectively. Acetogens provide double advantage of alternative H<sub>2</sub> and gut health for damage to the epithelium because of gut wear-out, a result of acidosis. In the hindgut, acetogenesis seems to be the main way for H<sub>2</sub> sink. For instance, Conrad [66] reported that in insect hindgut, acetogenesis is often the primary H<sub>2</sub>-consuming process, whereas, in gut lumen, methanogenesis seems to be limited to the intestinal walls and structures. It is important to note that gas production is inhibited when acetogenesis is the H<sub>2</sub> sink [14]. Use of acetogens or any strategy that can lead to increment of acetogens might reduce CH<sub>4</sub> production.

### 8.2. Propionate Acid Production

During starch fermentation, H<sub>2</sub> sinking enhances propionate acid production in the rumen [67]. Increasing propionate acid production through the use of grain in the diet of horses would have been a better option because of its ability to increase H<sub>2</sub> sinking, reduce methanogens through pH depression, which in turn, reduce or eliminate protozoa thereby inhibiting the H<sub>2</sub> exchange transfer between methanogens and protozoa. However, beyond CH<sub>4</sub> reduction, the health effect of prolonged depressed pH should be considered in animal, and a further consequence of endotoxin production and endotoxin absorption into the gut through the death of gram-negative bacteria.

### 8.3. Use of Biochar

*In vivo* study by Leng et al [59] showed that inclusion of biochar in digesta fermenting livestock diet reduced CH<sub>4</sub> production by 22%, and a combination of biochar and nitrate reduced CH<sub>4</sub> by 41%. The mechanism by which Biochar lowers CH<sub>4</sub> production is relating

**Table 1**  
Dietary treatments and additives to mitigate the methane emission in equine.

Dietary Treatment/Additive	CH <sub>4</sub> Reduction (%)	Impacts (Other Beneficial Impacts)	Reference
Cellulase, xylanase enzyme, and enzyme	23.4% in 24 h	3 μL/g DM resulted in greater gas production and improved fermentation kinetics	[56]
<i>Salix babylonica</i>	42.1% in 48 h and 21% in 72 h	Digestibility	[57]
Soybean hull	34.4% in 48 h and 16.1% in 72 h	Digestibility	[57]
Yeast	77%	Digestibility	[58]
Cellulase	42.0% in 24 h	Digestibility	[4]
Xylanase	35.7% in 24 h	Digestibility	[4]
Biocell F53	78% and 84.87% in 24 and 48 h, respectively		[58]
Biochar	22%	—	[62]

DM, dry matter.

to its insoluble component [59]. The principle behind the biochar reduction of CH<sub>4</sub> is its ability to absorb gasses because of its Brunauer–Emmett–Teller surface area which has about 2 to 4 m<sup>2</sup>/g biochar. Thus, inclusion of biochar in the diet of horses or hindgut fermenter might be a good option in reducing methanogenesis. This area requires further investigation. Therefore, inclusion of biochar in animal diet could be the affordable method for mitigating CH<sub>4</sub> production [14].

The mechanism behind this could be through stimulation of microbial growth, which reduces microbial cells and subsequently the substrate they use which serves as H<sub>2</sub> sink. Alternatively, it could also be that biochar stimulates the increase in microbial groups that oxidize CH<sub>4</sub>. Thus, inclusion of biochar alone or biochar with nitrate will reduce CH<sub>4</sub> production.

#### 8.4. Generation of Biogas Through Anaerobic Digester

Anaerobic digestion is means of recycling energy from plant biomass and the animal residues to reduce uncontrolled emissions of CH<sub>4</sub> during the storage and from landfills. Manure management is a challenge for many horse owners [68]. An average of 453.6 kg of horse produces 22.7 kg of raw waste per day if they are stall-fed, with the beddings adding about 4 to 7 kg more [69], and a million horses produce about 8 million tons of manure in a year [68,70]; in the face of limited arable land availability [71] for manure usage, anaerobic digestion might be a good option. Equine manure usually comes as mixture of feces and bedding material, and it is a good source of animal manure [46], which depicts that it contains reasonable number of nutrients in it. Consequently, stockpiling this manure in a way that gives room for anaerobic condition would lead to CH<sub>4</sub> emission from horse manure. However, their use is usually limited because of the fact that the bedding materials of horses are usually high in lignin and cellulose [72], and the lignin content limits the degradation of these materials. Besides, lignin and cellulose are in layers, and inability to degrade lignin will make cellulose inaccessible. Thus, using bedding material that is easily degradable might make horse manure a source of biogas material.

Although their dung may not be able to provide adequate micronutrient needed because it is usually lower than the requirement [73]. However, attention must be paid to season of harvesting the manure, as CH<sub>4</sub> output from horses is inconsistent, and the rate depends on the intensity of feeding the animal and the composition of forages fed to the animal [74] before they pass out the feces.

Cometabolism is a variation in anaerobic digestion system. This is a technique where more than one feces from two different animals (ruminant and nonruminant) are mixed together for digestion. This technique is aimed at increasing biodiversity [75], besides different microbial species degrade organic matter faster than individual microbes species; hence, the microbes metabolite

produced by one species would be used but other species [52], which would aid their proliferation and increase their degradation activity. Islas-Espinoza et al [72] demonstrated that inclusion of horse feces as inoculum in the anaerobic mixture of horse, sheep, and dogs' feces increases CH<sub>4</sub> output. Hence, anaerobic digestion of equine feces could be a good energy recovery mechanism that would otherwise have been returned to nature and become alternative source of farm income and greener environment if properly harnessed from economic point of view.

#### 8.5. Inclusion of Fibrolytic Enzymes

*In vitro* study of Salem et al [4] showed that inclusion of cellulase and xylanase at 2 μL/g dry matter (DM) reduced CH<sub>4</sub> produced by 78 and 55% compared with the control in horses. Furthermore, after 48 hours of incubation, cellulase and xylanase reduced CH<sub>4</sub> by 75 and 5.2% compared with the control. The plausible reason behind this is that the cellulase enzyme helped to increase cellulose digestibility/breakdown, which reduced the degradation time. Usually, diets that are low in digestibility stay longer in the gut than those that have higher digestibility, which is evident by the increase in feed intake when digestibility of feed is high. However, inclusion of cellulase enzyme in the diet of horses could help to reduce CH<sub>4</sub> output.

Kholif et al [56] showed that when the feces of horse that was fed diet was containing cellulase was used for *in vitro* study and cellulase was latter at 0, 1, and 3 μL/g DM, CH<sub>4</sub> production output was the lowest compared with the fecal source which contained xylanase, the control diet, and cellulase plus xylanase. One μL of enzymes/g DM produced the lowest CH<sub>4</sub> in 24 and 48 hours. Four milligrams per gram of DM of yeast cultures (Biocell F53) in [58] reduced CH<sub>4</sub> by 78.0 and 84.9% in 24 and 48 hours, respectively.

#### 8.6. Use of Plant Extract

In equines, handler, owner, and trainers use herbs for various purposes such as therapeutic or feed additives [76–79]. Plant extract confers different benefit such as improved performance, reduced CH<sub>4</sub> emission *in vitro* and *in vivo*, improved daily weight gain. The ability of plant extract to inhibit methanogenesis is based on the phytochemical compound in them which either reduce the methanogens activities or reduce protozoa population, thereby limiting the rate of H<sub>2</sub> exchange interaction between protozoa and methanogens. *In vitro* study of Elghandour et al [57] showed that inclusion of soya bean hull at 75 and 150 g/kg DM of total mixed ration, and *Salix babylonica* extract which is common in Mexico, at a rate of 0, 0.6, 1.2, and 1.8 mL/g DM of substrates reduced CH<sub>4</sub> output in 24 and 48 hours compared with the control. Inclusion of soybean hull at 75 g/kg DM of total mixed ration produced less CH<sub>4</sub> than soybean hull inclusion at 150 g/kg DM. Furthermore, under the lower soybean hull inclusion, 0.6 mL/g DM of *S. babylonica*

produced the least CH<sub>4</sub>, which was lower by 2.4-fold and 4.04-fold in 24 and 48 hours compared with the control. However, at the higher level of soybean hull inclusion, 1.2 mL/g DM produced the lowest CH<sub>4</sub> output compared with the control and other level of inclusion. Thus, it may be assumed that *S. babylonica* level should be increased when fiber is higher in diet.

Salem et al [5] incubated *Caesalpinia coriacea* and *Eichhornia crassipes* alongside *Avena sativa*, *Moringa oleifera*, and *Salix babylonica* at a rate of 0, 34, and 68 mg/g DM to determine the rate at which CH<sub>4</sub> gases will be produced. Compared with other plant inclusion, *Caesalpinia coriacea* produced the least CH<sub>4</sub> in all level of inclusion followed by *E. crassipes*. *C. coriacea* has (phenol and saponin in high quantity [5]), which would be responsible for the lower output. Indeed, plant secondary metabolites are important for the antimethanogenic activity/methanogenesis inhibition activity of plants and could be used a supplement in equine nutrition.

### 8.7. Chemical Method

Organic matter fermentation by methanogens occurs under same environment strictly devoid of oxygen and has a low redox potential condition, when nitrate and sulfate concentration is low [80]. Sulfate, nitrate, CO<sub>2</sub>, acetogenesis, and methanogens are compounds, and processes of natural H<sub>2</sub> sink in the animal. The variation in the functionally/efficacy of H<sub>2</sub> sinking by methanogenesis or acetogenesis is influenced by the partial pressure of H<sub>2</sub> gas in the biofilm or rumen, as methanogens have a lower threshold than acetogens with parts per million by volume of 6 to 120 [81] for methanogenesis, and acetogenesis is about 430 to 4,660 [14]. This gives methanogens advantage in H<sub>2</sub> sinking/potent greenhouse gases formation over acetogens. Importantly, this process is healthy for animals to help them maintain/continue fermentation processes and prevent gut distention, organ damage, or gut integrity compromise, but such process is deleterious to the atmosphere in its global warming potency. Cord-Ruwisch et al [82] reported that sulfate- or nitrate-reducing bacteria have the ability to lower H<sub>2</sub> partial pressure, and they have advantage over acetogens when competing with them for H<sub>2</sub>. Thus, inclusion of sulfate or nitrate in the diet of animals would be a good alternative to H<sub>2</sub> sinking than CO<sub>2</sub> [83].

## 9. Conclusion

Hindgut fermenter naturally produces less CH<sub>4</sub> compared with ruminant because of alternative H<sub>2</sub> sink that occurs in their gut. Although the contribution of equine to CH<sub>4</sub> seems negligible, however, increase in the population of horses worldwide would increase livestock contribution to CH<sub>4</sub>. Thus, the use of biochar, increase in acetogens, inclusion of fiber enzymes and plant extract, and recycling of fecal energy through anaerobic gas fermentation are feasible ways to reducing CH<sub>4</sub> contribution from horse and could be applied to ruminants too.

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