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## Chapter

# Review: Heads or Tails? Toward a Clear Role of Biochar as a Feed Additive on Ruminant's Methanogenesis

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## Abstract

The use of biochar has been suggested as a promising strategy in bio-waste management and greenhouse gases mitigation. Additionally, its use, as a feed additive, in ruminants has been reported to have contrasting effects on enteric methane production. Hence, this chapter intends to overview the most relevant literature that exploited the use of biochar as a mitigation strategy for methane. This includes the reported effects of biochar on methane production and rumen fermentation observed in *in vitro* and *in vivo* assays, as well as manure's methane emission. The information available about the biochar and the experimental conditions used in the different studies is still limited, which created additional challenges in identifying the biological mechanisms that potentially drive the contrasting results obtained. Nevertheless, it is clear from the current state-of-the-art that biochar may be a key player in the modulation of gut fermentation and in the reduction of greenhouse gases produced by ruminants that need to be consolidated by further research.

**Keywords:** biomass, biochar, enteric methane, *in vitro*, *in vivo*, ruminants

## 1. Introduction

The livestock sector was estimated to emit 14.5% of global anthropogenic greenhouse gases (GHG), mainly methane (CH<sub>4</sub>), nitrous oxide (N<sub>2</sub>O), and carbon dioxide (CO<sub>2</sub>) [1], with enteric CH<sub>4</sub> corresponding to 40% of total livestock sector emissions, 77% of which emitted by cattle [1].

Ruminants are herbivorous animals that host a complex symbiotic microbial population composed of bacteria, protozoa, archaea, fungi, and bacteriophages in the two forestomach (reticulum and rumen) where feeds undergo fermentation, before entering the true stomach, the abomasum. Microbial population ferments structural

and non-structural polysaccharides, and proteins originating volatile fatty acids (VFA) (mainly acetate, propionate, and butyrate), ammonia-N ( $\text{NH}_3\text{-N}$ ),  $\text{CO}_2$ , and hydrogen ( $\text{H}_2$ ) [2]. Volatile fatty acids are absorbed through the rumen wall and comprise the major energy source of the host animal. Hydrogen is mainly eliminated by the reduction of  $\text{CO}_2$  by methanogenic archaea [3]. Enteric  $\text{CH}_4$  represents a loss from 2 to 12% of total gross energy intake [4] and it is the second GHG contributor to climate change, with a global warming potential 28 times larger than  $\text{CO}_2$ , in a time horizon of 100 years. Mitigation of enteric  $\text{CH}_4$  emissions is thus important not only to minimize the environmental impact of ruminant production but also to improve feed efficiency.

Several strategies have been evaluated to reduce enteric  $\text{CH}_4$  production, including feeding management (e.g., ingredient selection, feed supplements, rate of passage, and better-quality ingredients), rumen modifiers (e.g., defaunation, bacteriocins, and immunization), and improvement of animal production through genetics (e.g., nutrient utilization, feed efficiency, and  $\text{CH}_4$  production) [5], but effects are often transient [6] or conflicting [7]. Greenhouse gases ( $\text{CH}_4$ ,  $\text{N}_2\text{O}$ ) and ammonia ( $\text{NH}_3$ ) are also produced during cattle manure decomposition in housing, storage, and treatment, and ultimately during land spreading [8]. Different strategies have been proposed to reduce gaseous emissions in each stage of manure management, from dietary manipulation to chemical application in slurry [9, 10]. One emerging strategy to cope with the mitigation of both enteric  $\text{CH}_4$  and GHG from ruminants' manure is the use of biochar. Biochar is a stable porous carbon-rich material (between 65 and 90%), mainly produced by the pyrolysis method under oxygen-limited conditions, containing mineral elements whose physical and chemical characteristics are determined by feedstocks and technologies involved in the production process [11, 12]. Due to its characteristics, biochar has been studied for multiple uses, such as soil amending [13–15], mitigating GHG emissions from soil [16–19], recovering nutrients from wastewaters [20], and reducing GHG emissions from cattle manure during storage [21, 22]. Its porous structure promotes soil moisture retention, reduces bulk density, enhances the organic matter content, and can positively affect soil cation exchange capacity [23, 24]. Due to these properties, interest has emerged in biochar as a feed supplement to mitigate enteric and fecal  $\text{CH}_4$ , and manure gaseous emissions [25, 26], in a cascade approach, thus enhancing its effect along the cattle production system [27]. In this context, the European biochar foundation has developed guidelines for biochar production to be used as a feed additive [28] under the requirements of the European Food Safety Authority (EFSA) and respecting the commission regulation (EC) 178/2002 [29] and 834/2007 [30].

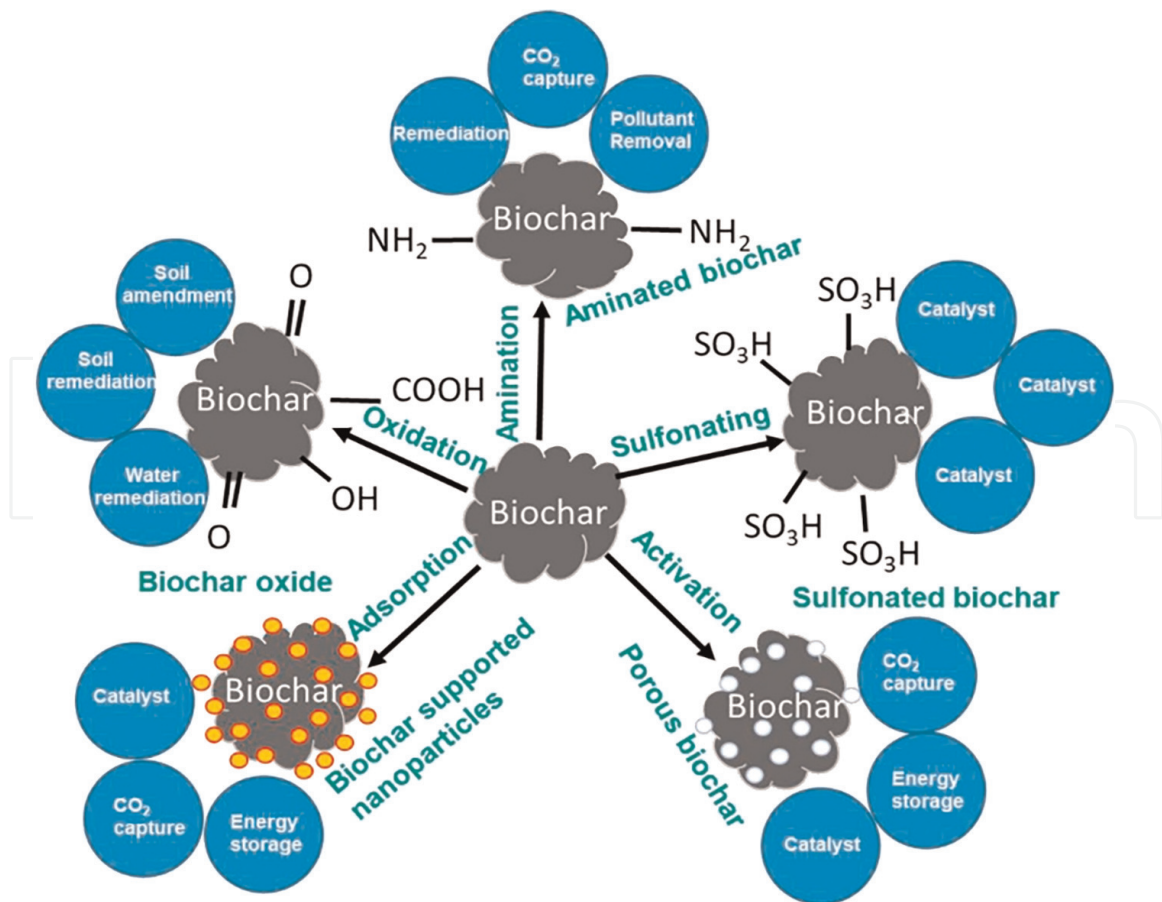
## **2. The role of biomass and production conditions on biochar characteristics**

The biomass source and the type and conditions of production are key factors in biochar physicochemical properties resulting in different functional characteristics and applications [31], being pyrolysis the most common process for the production of biochar. The characteristics of biochar can be highly variable, especially in terms of elemental composition, surface chemical composition, structure, and stability. Each component's decomposition and depolymerization occurs through several reactions at different temperatures, contributing to the structural differences among biochars [32, 33].

In the works reviewed here, biochars were mainly produced by the pyrolysis of agriculture and forestry lignocellulosic biomasses, which are primarily composed of cellulose (40–45%), hemicellulose (25–35%) and lignin (20–30%), although their distribution varies among biomasses [34]. In terms of gaseous capture, the most relevant characteristics of biochar are the organic matter content (given by polarity and aromaticity), mineral content, cation exchange capacity, surface charge, and textural properties (surface area and pore size) [35].

The adsorption capacity of biochar related to the polarity and aromaticity is highly modulated by the pyrolysis conditions [35]. Due to the high carbon content and porous structure, adsorption is a valuable property of biochar, which has been used for environmental purposes, such as the reduction of GHG levels [35]. Therefore, the physical-chemical characteristics of the biochar have a strong influence on the capabilities of the materials for a particular application (**Figure 1**).

For example, the ash content that results from the decomposition of the inorganic matter of biomass [23] is expected to be low in wood-based biomass when compared to mineral-rich biomass, such as grass, manure, litter, and solid waste [36]. Wood, bamboo, corncob, corn stover, pellets (miscanthus, softwood, wheat straw, and oil-seed rape straw), rice straw, and potato peel biochar reported less than 25% of ash content, while rice husk presented higher than 40% [37–44]. The ash content has been demonstrated to be relevant for the surface polarity and distribution of pores, thus influencing the sorption capacity of the material. The mineral content in biochar (such as carbonates, oxides, phosphates, alkali, or alkaline earth metals) has been shown to



**Figure 1.** Biochar post-production functionalization and potential applications. Reprinted with permission from Ghodake et al. [33].



increase the sorption capacity for acidic gases, such as sulfur dioxide, hydrogen sulfide, and CO<sub>2</sub> [12].

The surface area and pore size can also be modified by chemical and physical activation following the carbonization process [45]. The modification of biochar with a CO<sub>2</sub>-NH<sub>3</sub> mixture resulted in a surface area increase besides improving the chemical properties of the surface by a nitrogen modification [46]. The microporous structure has a key role in CO<sub>2</sub> capture at low temperatures [47].

Using lignocellulosic biomasses (the main raw material present in the application herein described), a microporous structure is expected with higher cellulose and hemicellulose content, whereas mesoporous structures are expected with higher lignin content [27]. The increase in pyrolysis temperatures also increases the porosity, surface area, pH, ash, and carbon content of biochar due to the release of volatile components, while reducing biochar exchange capacity and yield [20, 48]. In the study of Calvelo Pereira *et al.* [38], an increase in surface area, carbon, nitrogen, and ash contents of biochar produced from the pyrolysis of pine chips and corn stover was observed. Also, biomass has been shown to highly influence the surface area, as demonstrated by other authors [40, 44].

### 3. Effects of biochar on *in vitro* rumen fermentation

There is a paucity of data on the effects of biochar on CH<sub>4</sub> production by short- and long-term *in vitro* studies. Therefore, these will be addressed separately.

#### 3.1 *In vitro* short-term studies

**Table 1** presents the results obtained in 14 studies evaluating the effects of biochar addition up to 16% on rumen fermentation and CH<sub>4</sub> production through *in vitro* short-term incubations (up to 48 h). No clear association is evident between effects on CH<sub>4</sub> production and biochar characteristics (e.g., biomass, temperature of pyrolysis) and level of inclusion. Increasing pyrolysis temperature increases surface area, which has the potential to improve biofilm formation and promote the adsorption capacity of microorganisms, nutrients, and gases, thus reducing CH<sub>4</sub> production [26, 49]. Indeed, some studies [44, 50–54] reported a decrease in CH<sub>4</sub> production with the addition of biochar produced at very high temperatures (700–1000°C), whereas in the studies using biochar produced at lower temperatures (350–700°C) no effect [38, 55] or an increased [42] CH<sub>4</sub> production was observed. However, Saenab *et al.* [56] reported a decrease in CH<sub>4</sub> production when biochar from cashew nutshell was produced at 300°C and Cabeza *et al.* [40] found higher CH<sub>4</sub> production with biochar produced at 700°C than 550°C. It must be realized that *in vitro* systems do not effectively reproduce the *in vivo* situation, particularly the adaptation of rumen microbiome to novel materials, and for this reason, effects *in vitro* might not be observed *in vivo* [5].

The information about biochar characteristics (besides pyrolysis temperature), is absent in the majority of the studies, making impossible any association between the results and the biochar characteristics and their respective effects on CH<sub>4</sub> production. Despite not having evaluated the effect on CH<sub>4</sub> mitigation, McFarlane *et al.* [39] found biochar particle size to affect rumen fermentation, being inhibited with large particles (>178 µm *vs.* <178 µm). Although without impact on gas production and

Biomass	Temperature	Time (h)	Incubation level	CH <sub>4</sub> production	Reference
Rice husk	900–1000	24	0, 1, 2, 3, 4, 5	↓ with 1% biochar; no further benefits with 2–5% biochar	[51]
		24	0, 0.5, 1	↓ with 0.5 and 1%; further reductions with addition of nitrate N and urea	[51]
		24	1.5	↓ with adapted inoculum or biochar addition	[57]
	700–900	24, 48	1	↓ with higher reduction at 48 h	[50]
	1000	24	1	↓	[53]
	1000	6, 12, 18, 24	1	↓ at 18–24h	[54]
Pine wood chips	350	2, 6, 12, 24	16	Not affected	[38]
	550				
Corn stover	350				
	550				
Gasified	—	48	9	Not affected	[37]
Straw-based					
Wood-based					
Activated carbon					
Miscanthus straw pellets	550	24	1	↓ with biochar over the control;	[40]
	700		10	No differences between sources;	
Oilseed rape straw pellets	550		1	↑ with 700°C over 550°C	
	700		10		
Rice husk	550		1		
	700		10		
Cashew nutshell	300	3, 6, 9, 12, 24, 30, 36, 48	0, 0.75, 1.5, 2.25, 3	↓ with biochar; ↓ ↓ with biochar and bio fat	[56]
Potato peel	500	24	0, 5, 10	↑ over the control	[42]
Agro-forestry	600	24			
Mixed species of green waste tree pruning	500	6, 12, 24	0, 0.5, 1, 2, 4	Not affected by inclusion level	[55]
Rice straw	300, 500, 700	4, 24, 48	3	↓ with rice straw and corncob in comparison to bamboo at 4 and 48 h;	[44]
Corncob				↓ with increasing temperature	
Bamboo					

**Table 1.**  
*Biochar biomass, temperature of pyrolysis (°C), and inclusion level (% dry matter basis) effects on methane (CH<sub>4</sub>) production in short-term in vitro studies.*

VFA proportions, these authors reported *in vitro* true digestibility of orchard grass hay to be increased by the inclusion of fine biochar particle size [39].

A comparison between studies is further complicated by the diversity of biomass sources used (e.g., rice husk, pine wood, corn stover, cashew nutshell, tree pruning, rice straw, corncob, bamboo) that might affect VFA profile, thus introducing a confounding effect on the mechanism of CH<sub>4</sub> reduction. Most studies that compared the impact of biomass sources on enteric CH<sub>4</sub> production [37, 38, 41, 42] observed no differences among biochar sources. Conversely, Van Dung *et al.* [44] found rice straw and bamboo biomass to reduce CH<sub>4</sub> production compared to corncob, at 4 and 48 h of incubation, but not at 24 h. Moreover, these authors observed an interaction effect between biomass source and pyrolysis temperature [44], supporting the need for a multi-aspect analysis of biochar's chemical and physical properties. The effects on VFA profile were further assessed [38, 40, 42, 55, 56]. In the study of Calvelo Pereira *et al.* [38], despite a decrease in propionate proportion found with some mixtures, which might indicate an increase in H<sub>2</sub> produced, the effects were insufficient to affect CH<sub>4</sub> production. In the study by Cabeza *et al.* [40], the addition of biochar slightly reduced CH<sub>4</sub> production, but it kept unchanged the amounts of total VFA or acetate produced and reduced those of propionate and butyrate. Saenab *et al.* [56] observed a reduction of CH<sub>4</sub> production by 11.5% with 3% [dry matter (DM) basis] cashew nutshell biochar supplementation, although total and individual VFA produced were unaffected. Rodrigues *et al.* [42] attributed the reduction of VFA production through biochar addition to a reduced energy supply for microbial growth. Supplementation of tree pruning biochar up to 4% (DM basis) did not affect CH<sub>4</sub> or VFA content and profile [55].

The study by Leng *et al.* [57] was the only one that evaluated the effect of rumen fluid adapted to biochar. The authors attributed the reduction in CH<sub>4</sub> production with rumen-adapted inoculum to a larger ruminal population that oxidizes CH<sub>4</sub>. Indeed, adapted rumen inoculum is expected to present a higher density of methanotrophs [58], possible the effect of biochar on rumen CH<sub>4</sub> is solely due to the increase in potential habitat for this consortium. However, in the study by Leng *et al.* [57], CH<sub>4</sub> reduction was higher with biochar addition to unadapted rumen inoculum than without biochar addition to adapted rumen inoculum. Biochar addition promotes either the association of microorganisms that more efficiently ferment feed materials or facilitates CH<sub>4</sub> oxidation by bringing together methanogenic archaea and methanotrophic consortia [59].

However, from the available studies, the mechanism of CH<sub>4</sub> reduction through biochar is unclear. Although biochar favors methanotrophism in the soil [60], the anaerobic rumen precludes the growth of aerobic methanotrophs, thus the action of biochar is most possibly through the promotion of micro-environments by the large surface area of biochar [40].

### 3.2 *In vitro* long-term studies

The long-term effects of biochar supplementation on rumen fermentation and CH<sub>4</sub> production were further assessed *in vitro* using the rumen simulation technique system (**Table 2**). Despite differences among biochar biomass, pyrolysis temperature, and chemical and physical characteristics, only one study observed a CH<sub>4</sub> mitigation effect of biochar when compared to control [41]; supplementation levels (0.5, 1, and 2%, DM basis) having a quadratic effect, greatest with 0.5% inclusion. Jackpine biochar also improved most fermentation parameters (e.g., NH<sub>3</sub>-N, total VFA,

Biomass	Temperature	Inclusion level	Substrate	Effects	Reference
Jackpine	600	0, 0.5, 1, 2	Barley silage: rolled barley grain: canola meal: concentrate (60:27:10:3)	Compared to control; ↓ CH <sub>4</sub> and ↑ VFA; = gas, pH, protozoa; linearly ↑ NH <sub>3</sub> -N, DMD, CPD, NDFD, ADFD, total and LAB microbial N	[44]
Hardwood blackbutt, clay, and minerals	650	0, 3.6, 7.2	Oaten pasture: maize silage: concentrate (35:35:30)	= CH <sub>4</sub> and total gas, pH, NH <sub>3</sub> - N, VFA, DMD, microbial richness, and diversity; 7.2% tended to ↓ CH <sub>4</sub> compared to 3.6%	[61]
Spruce steem	450	2	Barley silage: rolled barley grain: canola meal: premix (60:27:10:3)	Tended to ↓ CH <sub>4</sub> (% total gas); = total gas, pH, VFA, protozoa, microbial N, bacterial richness, diversity, and relative abundance	[62]
Jackpine/ yellow pine	400–600	2	Barley silage: rolled barley grain: canola meal: premix (60:27:10:3)	= CH <sub>4</sub> , total gas, pH, VFA, protozoa, microbial N, bacterial richness, diversity, and relative abundance	[43]

NH<sub>3</sub>-N- ammonia-N, DMD- dry matter digestibility, CPD- crude protein digestibility, NDFD- neutral detergent fiber digestibility, ADFD- acid detergent fiber, LAB- liquid associated bacteria, and VFA- volatile fatty acids.

**Table 2.**  
*Biochar biomass, temperature of pyrolysis (°C), and inclusion levels (% dry matter basis) effects on rumen fermentation and methane (CH<sub>4</sub>) production in long-term in vitro studies.*

acetate, propionate, butyrate, and branched-chain VFA yield), nutrient digestibility (DM, crude protein, neutral detergent fiber, acid detergent fiber), and microbial N of total and liquid associated bacteria while decreased that of loosely associated bacteria [41]. Conversely, mineral-activated blackbutt [61], jack/yellow pine [43] and spruce stem [62] biochar supplementation kept unaffected gas production, fermentation parameters (pH, NH<sub>3</sub>-N, total and individual VFA yield), nutrient digestibility, microbial N produced, protozoa count, and bacterial diversity, richness, and relative abundance. Inconsistency of biochar effects has been attributed to variations in biochar chemical and physical properties, including particle size, adsorptive potential, electrical conductivity, and electron-mediation in redox reactions [37, 39]. Several modification methods have been used to improve biochar properties, such as acidification of surface area, to increase biochar adsorption [23]. Teoh *et al.* [61] further suggested that biochar pH could be of particular importance in enteric CH<sub>4</sub> reduction, based on the notable CH<sub>4</sub> reduction (25%, as mg/g DM incubated) of the acidic (pH 4.8) jack pine biochar used in Saleem *et al.* [41] study. Acidic biochar has been associated with improved carbon sequestrum and higher redox potential in soils, whereas neutral mineral-rich biochar lacked this ability [63]. However, acidic (pH 4.9) pine biochar failed to reduce enteric CH<sub>4</sub> production [43] similarly to observed with basic (pH 8.2) biochar supplementation [37, 38, 61].

Acidic biochar has also been suggested to improve the redox potential and thus increase biofilm development by the mediation of electrons among the microbial population [61, 64]. However, more developed biofilms were observed on readily digestible



substrates than on biochar surfaces [62, 65]. Even though microbial diversity, richness, and relative abundance were not affected by long-term biochar supplementation, discriminant analysis unveiled biochar-type specific changes in rumen bacterial families [43, 61, 62]. Of particular interest, Teoh *et al.* [61] found a 19.8-fold reduction in the abundance of *Methanomethylophilaceae* with the supplementation of mineral-activated biochar. Members of *Methanomethylophilaceae* family are methanogenic archaea that use sources of hydrogen to reduce methylated compounds and produce CH<sub>4</sub> [66, 67], thus suggesting the potential mitigation effect of hardwood biochar [61].

4. Effects of biochar *in vivo*

The porous structure of biochar can adsorb gases and provide habitat for microbial biofilms [37, 68], which in addition to electron-mediation properties in biological redox reactions [69] suggest its potential to reduce enteric CH<sub>4</sub> production and promote rumen fermentation. As previously stated, *in vitro* studies present several advantages, but do not fully simulate the *in vivo* animal. Few studies have evaluated, *in vivo*, the effects of dietary biochar inclusion on ruminant performance and CH<sub>4</sub> production (Table 3). Globally, dietary supplementation with biochar from different sources increased or not affected ruminant performance and reduced or kept

Animals	Diet	Biochar level (source)	Observations	Reference
Cattle (80–100 kg)	Cassava root chips and fresh cassava foliage	0.6 (rice husks)	Live weight gain ↑ 25%; ↑ DM feed conversion; ↓ CH <sub>4</sub> production	[52]
Angus × Hereford heifers (565 ± 35 kg)	Barley silage-based diet	0, 0.5, 1, 2 (pine-enhanced biochar)	CH <sub>4</sub> emissions not affected; Specific rumen microbiota altered	[65]
Crossbred steers (529 ± 16 kg)	Growing diet: brome hay: wheat straw: corn silage: wet distillers’ grains: supplement (21:20:30:22:7) Finishing diet: dry-rolled corn: corn silage: wet distillers: supplement (53:15:25:7)	0, 0.8, 3 (whole pine trees)	CH <sub>4</sub> tended to decrease in the growing animals; CH <sub>4</sub> is not affected in the finishing animals	[70]
Lambs (37.9 ± 0.8 kg)	Alfalfa and barley (60:40) <i>ad libitum</i>	0, 2 (Lodgepole pine and quaking aspen)	= feed intake and average daily gain; ↑ DM digestibility and digestible DM intake	[71]
Kermanian ram lambs (21.9 ± 2.24 kg)	Alfalfa: wheat straw: concentrate (30:10:60)	0, 1, 1.5 (Walnut shell and pistachio by-product at 1%, chicken manure at 1.5%)	= DM intake; ↑ average daily gain; ↑ feed conversion ratio	[72]
<i>Bos taurus</i> crossbred beef steers initial (286 ± 26 kg)	High-forage and high-grain diets	0, 0.5, 1, 2 (Yellow pine)	2% lean meat yield; = body weight and DM intake	[73]

Animals	Diet	Biochar level (source)	Observations	Reference
Milking dairy cows	Barley hay and compound (40:6) free-access to forage during the day	0.5 (powdered activated carbon)	↓ manure CH <sub>4</sub> by 30–40% and CO <sub>2</sub> emissions by 10%; ↑ milk production; ↓ manure methanogenic flora by 30%; ↑ nonmethanogenic species	[74]
DM- dry matter, and CO <sub>2</sub> - carbon dioxide.				

**Table 3.**  
Effect of biochar biomass and inclusion level (% dry matter basis) on ruminant performance and methane (CH<sub>4</sub>) production.

unaffected CH<sub>4</sub> production. Leng *et al.* [52] pointed out the need for CH<sub>4</sub> mitigation strategies to include alternative electron sinks rather than just focused on methanogens inhibition, due to the need for symbiotic associations in biofilm microbial colonies on feed particles for successful ruminal fermentation to occur. Rumen microbial biofilms are of particular importance for fiber fermentation, with microbial attachment to feed particles allowing pit formation as well as glycocalyx emission to fibrous amorphous material [75].

In Angus × Hereford heifers, Terry *et al.* [65] found that, although total tract digestibility, nitrogen balance, and CH<sub>4</sub> production were not affected by dietary biochar inclusion, the relative abundance of *Fibrobacter* and *Tenericutes* were reduced and that of *Spirochaetaes*, *Verrucomicrobia*, and *Elusimicrobia* increased. Modulation of the manure microbial population was also found to be affected by dietary biochar supplementation. Al-Azzawi *et al.* [74] reported decreased methanogenic population by 30% with a corresponding increase in the non-methanogenic archaeal species in manure, suggesting that formed CH<sub>4</sub> could be reduced by further utilization by methanotrophic species. Moreover, biochar was shown to affect nitrification by increasing ammonia-oxidizing organisms and reducing ammonooxygenase activity [76].

Although dietary biochar supplementation had variable effects on ruminant performance, these were overall promising and suggest potential benefits beyond methanogenesis. Indeed, 0.6% biochar increased the live weight gain of yellow cattle and DM feed conversion by 25% [52]. Terry *et al.* [73] found no effect on body weight gain or DM intake in beef steers up to 2% biochar, but lean meat yield increased with the highest biochar level tested (2%). In lambs, 2% biochar kept feed intake and average daily gain unaffected, and improved DM intake [71], while up to 1.5% biochar was found to maintain DM intake and increase average daily gain and feed conversion ratio [72]. In addition, milk production of cows fed 0.5% (DM basis) activated carbon was improved [74]. Furthermore, in an innovative solution for biochar utilization reported by Joseph *et al.* [77], biochar was mixed with molasses and fed directly to cows, the dung-biochar mixture being incorporated into the soil profile by dung beetles and the costs and benefits of integrating biochar with animal husbandry and improvement of pastures were assessed. These authors found that dung-biochar had an outer coating of mineral elements (P, K, Mg, Ca, Al, Si, and Fe) and nitrogen, adsorbed in the cow gut, that were available for soil, thus being an effective strategy to improve soil properties. In addition, increasing returns to farmers were calculated, suggesting the profitability of dietary biochar supplementation in ruminant production systems [77].

Notwithstanding, the inconsistent results in the literature on the effect of biochar on reducing CH<sub>4</sub> emissions, rumen *in vitro* fermentation, and *in vivo* rumen function limits the mechanistic understanding of the underlying mode of action. This is particularly difficult due to the use of different sources of biomass and production conditions, such as duration and temperature, of pyrolysis as well as post-treatment modifications, which alter the composition, porosity, and chemistry of biochar [65], but also to the poorly characterized biochar used in ruminant studies. These challenges make comparisons between studies difficult, and in addition to the lack of knowledge of the long-term effects of dietary biochar supplementation, could have limited its use in ruminant feeding practices on-farm.

## 5. Effects of biochar on manure CH<sub>4</sub> production

Ruminant production generates high amounts of manure that need to be stored until the land application. Manure is a rich source of nutrients, and its application is shown to improve soil quality, to reduce the use of mineral fertilizers and costs of production [21]. However, during manure storage and land application, malodorous compounds and GHG, such as CH<sub>4</sub>, CO<sub>2</sub>, and N<sub>2</sub>O, as well as NH<sub>3</sub>, are formed and emitted [78], with a detrimental impact on ecosystems [22]. Biochar application to manure can be an effective strategy to improve its environmental impact, as it can absorb and retain GHG, NH<sub>3</sub>, and nutrients [79, 80]. Moreover, when applied to soils, biochar-enriched manure may provide nutrients, sequester carbon, and improve soil's structure [22, 79]. Although the already identified biochar potential in manure, differences have been reported among biochar biomass, production conditions, pH, hydrophobicity, and particle size [22, 68, 81]. Moreover, a life cycle assessment of the environmental implications of stored cattle slurry (a mixture of manure, split feed, and water) treatments revealed biochar to be one of the less effective approaches to suppress GHG emissions from liquid slurry, except for N<sub>2</sub>O [21]. The inconsistent results from biochar application to manure pinpoint the need for more research in this field.

## 6. Conclusions

Biochar is undoubtedly a material with high potential to deal with ruminant methanogenesis due to its availability, stability, and large surface areas. Nevertheless, there is a significant knowledge gap about the mechanisms that govern the interactions between biochar and the plethora of microorganisms that are present in the ruminant's gut and manure. In this chapter, we addressed the most relevant literature on the topic, seeking additional clarification about the potential role of biochar in methanogenesis. The absence of detailed characterization of biochar used, and the diversity of the experimental conditions applied in the different studies, create additional challenges for a critical comparison of the past findings. Therefore, for future studies, some level of standardization and the detailed characterization of the biochar(s) used will have a significant impact on the clarification of its role in the mitigation of GHG emissions from ruminants.

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## **Conflict of interest**

The authors declare no conflict of interest.

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