STRATEGIES TO REDUCE METHANE EMISSIONS FROM ENTERIC AND LAGOON SOURCES

(Contract 17RD018)

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DISCLAIMER

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List of Abbreviations

3NOP	3-nitrooxypropanol
BW	Body weight
CARB	California Air Resources Board
CDFA	California Department of Food and Agriculture
CP	Crude protein (% dry matter)
DDGS	Dry distillers grain with solubles
DMI	Dry matter intake (kg/d)
ECM	Energy corrected milk
GE	Gross energy
GHG	Greenhouse gases
IPCC	Intergovernmental Panel for Climate Change
LCA	Life cycle assessment
MD	Mean difference
NDF	Neutral detergent fiber (% dry matter)
OM	Organic matter)
RMD	Relative mean difference
RVE	robust variance estimation
SMD	Standardized mean difference
USDA	United States Department of Agriculture
USDA NASS	United States Department of Agriculture National Agricultural Statistics Service
USDA ERS	United States Department of Agriculture Economic Research Service

ABSTRACT

The State of California launched the short-lived climate pollutant reduction strategy (SB 1383) with the objective of decreasing methane (CH_4) emissions from livestock by 40% by 2030 from 2013 levels. Considering about 50% of CH_4 emissions in the State are attributed to enteric fermentation and manure, achieving significant CH_4 emission reduction from these sources will be critical to meeting SB1383 goals. There are numerous mitigation options described in the literature including feed and manure additives. The objective of the study was to provide quantitative analysis, evaluate feasibility, and summarize and prioritize research gaps to guide future research in the State. Specifically the current study conducted a literature review of available mitigation strategies using additives to reduce enteric and manure methane emissions including size effect and performance analyses and used life-cycle assessment tools to estimate net greenhouse gas emissions from using potential feed additives in the dairy industry. Effect size and meta-analyses were conducted to identify the additives with greatest potential for CH_4 mitigation. For feed additives, 3-nitrooxypropanol (3NOP), bromochloromethane, chestnut, coconut, distillers dried grains and solubles, eugenol, grape pomace, linseed, monensin, nitrate, nitroethane, saifoin, fumaric acid, and tannins had significant impacts on enteric emissions. For manure additives, acidification, biochar, microbial digestion, physical agents, straw, and other chemicals significantly reduced CH_4 emissions. However, there were other promising additives that need further research, including Mootral, macroalgae and SOP lagoon additive (SOP). After further analysis of variance, the most effective feed additives were 3NOP (41% in dairy and 22% reduction in beef) and nitrate (14.4% reduction). Biochar as a manure additive can be effective on compost manure (up to 82.4% reduction), but may have no impact on lagoon emissions. A life cycle assessment tool was used to estimate the net reduction in enteric CH_4 emissions by

using the feed additives 3NOP and nitrate. The overall average net reduction rate of supplementing 3NOP and nitrate were 11.7% and 4.9%, respectively. Given the toxicity concerns of nitrate, only 3NOP is recommended for use pending FDA approval. Considering California milk production of 18 billion kg in 2017, using nitrate on California dairy cows would reduce GHG emissions 1.09 billion kg CO_2e and 3NOP 2.33 billion kg CO_2e annually. Further research in the additives of Mootral, macroalage, SOP, biochar and other emerging ones is required before recommendation for use can be made.

EXECUTIVE SUMMARY

Background

About 50% of CH_4 emissions in California are attributed to enteric fermentation and manure; therefore, achieving significant methane (CH_4) emission reduction from these sources will be critical to meeting SB1383 goals. There are several strategies for reducing CH_4 emissions from enteric fermentation and manure management in the literature (e.g., Knapp et al. 2014), including diet manipulation, feed additives, anaerobic digestion and liquid-solid separation. A number of excellent reviews on enteric methane mitigation techniques have already been published. Similarly, there are a number of reviews available that summarize mitigation options to reduce CH_4 emissions from manure management. However, none of the reviews quantitatively evaluate impact of feed and manure additives in a meta-analytic and holistic manner. The overall objective of this study was to review feed and manure additives used for CH_4 emission reduction and identify those with the potential to be applied in the California livestock industry. The feed and manure additives were classified into three categories as follows. Category 1: Safe and effective for methane use, recommended when all regulatory approvals are in place. Category 2: Research to date shows this product may be effective and more research is required before it is recommended for use. Category 3: Research to date has either provided insufficient evidence to conclude that the product may be effective, or has shown that product is not effective, or has shown that the product should not be used for other reasons.

Methods

Extensive literature survey on feed and manure additives was conducted and data collected in an excel spreadsheet that includes information on methane emissions as well as dietary and other factors. Effect size estimates of mean difference (MD; i.e., mean treatment minus mean control) and standardized mean difference (SMD) were calculated using the open source statistical software R (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria). For some feed and manure additives, a meta-analysis was conducted using the robust variance estimation method to deal with unknown correlations among non-independent effect sizes. For the most promising feed additives, a life cycle assessment approach was taken in which crop production, additive production, farm operation, enteric emissions, and manure emissions were taken into account to estimate the net greenhouse gas emission in producing a kilogram of milk.

Results

A literature survey of feed additives with anti-methanogenic properties revealed over 90 potential additives. However, after analyzing their impact on CH_4 emissions only 3-nitrooxypropanol (3NOP), bromochloromethane, chestnut, coconut, DDGS, eugenol, grape pomace or marc, linseed, monensin, nitrate, nitroethane, saifoin, fumaric acid, and tannins had overall CH_4 reduction potential. Of these, only 3NOP and nitrate were considered to have the best potential outcome for mitigation. Feed additives such as Mootral, macroalgae and Agolin have also shown promise but there is limited *in vivo* work to allow full consideration. A total of 13 categories of manure additives were included for their potential to reduce emissions. In a meta-analysis, acidification, biochar, microbial digestion, physical agents, straw, and other chemicals significantly reduced CH_4 emissions. The most effective manure additive was biochar, which significantly reduced CH_4 emissions by up to 82.4%. However, further work is needed to develop a protocol on the type/dose of biochar and its effectiveness based environmental conditions. Other manure additives were not included in the analysis because only one or two experiments have been conducted (e.g. SOP; Borgonovo et al., 2019). It has a potential but needs further study. The two promising feed additives that been research extensively were further evaluated using a life cycle assessment tool to estimate their net reduction potential from dairy systems in California by considering their impact on other parts of the industry as well as environmental cost of additive production. The average net reduction rate of supplementing 3NOP and nitrate were 11.7% and 4.9%, respectively. 3NOP had a greater effect than nitrate on reducing total GHG emissions with a highest performance of 11.8%. Feeding 3NOP to only lactating cows or to the entire growth stages did not make significant difference in total GHG emissions. Considering California milk production of 18 billion kg in 2017, using nitrate on California dairy cows would reduce GHG emissions by 1.09 billion kg CO_2e and 3NOP by 2.33 billion kg CO_2e annually. Unless the toxic effect of nitrate at high doses are mitigated, nitrate is not recommended at present.

Conclusion

At the writing of the report, we recommend 3NOP to be in Category 1 with the highest potential impact pending FDA approval. Nitrate (if toxicity mitigated), Mootral, macroalgae, Agolin and grape pomace are recommended to be in Category 2 with further experiments required to verify the impact already shown in California. The rest should be in Category 3, which include additives not recommended at this time. For manure additives, biochar is in Category 1 with the caveat already mentioned above. Acidification and SOP manure additive are in Category 2, which need further study. Most of the research for biochar and straw is when used as additive to solid or semi solid manure so they should be interpreted in that context.

INTRODUCTION

Global emissions of greenhouse gases (GHG) have risen to unprecedented levels despite a growing number of policies to reduce climate change (IPCC, 2014). Anthropogenic sources account for 58% of global GHG emissions (EPA, 2011), 18% (5.0 – 5.8 Gt CO2eq /yr) of which was generated by agriculture-related activities during 2000–2010 period (Smith et al., 2014). Methane (CH_4) from enteric fermentation and manure was the largest contributor (40%) to the agricultural GHG emissions (Tubiello et al., 2013). The largest source of anthropogenic CH_4 in the US is from livestock, particularly ruminants (EPA, 2017).

The State of California launched the short-lived climate pollutant reduction strategy (SB 1383; CARB 2017) with the objective of decreasing CH_4 emissions from livestock by 40% by 2030 from 2013 levels. About 50% of CH_4 emissions in the State are attributed to enteric fermentation and manure (CARB, 2020); therefore, achieving significant CH_4 emission reduction from these sources will be critical to meeting SB1383 goals. There are several strategies for reducing CH_4 emissions from enteric fermentation and manure management in the literature (e.g., Knapp et al. 2014), including diet manipulation, feed additives, anaerobic digestion and liquid-solid separation. This proposal is focused on additives that reduce methane emissions from enteric and lagoon sources.

A number of excellent reviews on enteric methane mitigation techniques have already been published (e.g., Boadi et al., 2004; Beauchemin et al., 2009; Cottle et al., 2011; Hristov et al., 2013). Similarly, there are a number of reviews available that summarize mitigation options to reduce CH_4 emissions from manure management (e.g., Kebreab et al., 2006; Jayasundara et al., 2016). Recently, an international group of scientists (including the PI) conducted a comprehensive analysis of mitigation options for reducing enteric (Hristov et al., 2013a) and manure (Montes et al., 2013) emissions. The intention of this proposed study is not to reproduce them but to evaluate statistically the effectiveness of various mitigation techniques. Studies on novel feed additives have been published recently and continue to be reported in the literature, which may have not been included in the previously mentioned reviews. There is a need for a comprehensive review and analysis of additives that have the potential to be successful in California in mitigating emissions. The review will take a holistic approach and extend to include a life-cycle analysis (LCA) of the impact of additives. This will allow a fuller environmental impact assessment, which is associated with implementing some of the additives that have already been developed and some that are currently being tested.

The overall objective of this study is to review feed and manure additives used for methane emission reduction and identify/categorize those with the potential to be applied in the California livestock industry. The strategies will be analyzed not only for their potential to reduce emissions but also their impact, if any, on product quality and animal welfare. Analysis of additives for methane mitigation potential will take a life-cycle approach, which will be required in case production and implementation of additives will have upstream and downstream consequences that may change the net benefit. The additives will be placed into the following three categories: Category 1: Safe and effective for methane use, recommended when all regulatory approvals are in place. Category 2: Research to date shows this product may be effective and more research is required before it is recommended for use. Category 3: Research to date has either provided insufficient evidence to conclude that the product may be effective, or has shown that product is not effective, or has shown that the product should not be used for other reasons. The ultimate objective is to provide quantitative analysis, summarize and prioritize research gaps to guide future research in the State. The following specific objectives will be addressed in the current study:

1. Literature review of available mitigation strategies using additives to reduce enteric and manure methane emissions including size effect and performance analyses.

2. Prioritize research gaps and use life-cycle analysis to assess potential unintended impacts such as greater emission in sourcing the product or product development.

FEED ADDITIVES TARGETING ENTERIC METHANE EMISSIONS

A literature survey of feed additives used targeting enteric methane emissions was conducted. There were a total of 90 different feed additives collected from the literature. The counts of treatment, averages, standard deviations, minimums and maximums of Mean Difference (i.e., mean treatment minus mean control) of CH_4 production for control/treatment groups based on feed additive type is summarized in Appendix 1. Methane production and methane production per dry matter intake (DMI) were expressed in g/day and g/kg, respectively. Effect size estimates of mean difference (MD) and standardized mean difference (SMD) were calculated using the open source statistical software R (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria). Any feed additive related studies without CH_4 production information listed in the database were excluded in the further analysis. Furthermore, feed additives with only one record were excluded in further tests because lack of replications prevents the calculation of standard deviation, and *P*-values.

After the data was filtered and selected based on the criteria mentioned above, a sample *t*-test (treatment-control) was conducted based for each feed additive. Table 1 gives the *P*-values from significant *t*-test (α=0.05). As a result, feed additives including 3-nitrooxypropanol (3NOP), bromochloromethane, chestnut, coconut inclusion, DDGS concentrate, eugenol, grape pomace, linseed, monensin, nitrate, nitroethane, saifoin, fumaric acid, hydrolysable tannins, and *Sericea lespedeza* tannins significantly impacted the MD of CH_4 production. Similarly, a box and forest plots were constructed to assess the impact of feed additives on methane production (Fig. 1, 2).

Table 1. Impact of feed additives on CH_4 reductions (g/d) based on *t*-test.

Feed additive*	***P*-value**	**Feed additive**	***P*-value**
3NOP	*0.002*	Legume	0.403
Acacia mearnsii	0.057	Linseed inclusion	*0.007*
Acetate inclusion	0.084	Lotus tannins	0.989
Microbial culture	0.051	Lovastatin	0.130
Bromochloromethane	*<0.001*	Lupine seed	0.449
Calcium soap inclusion	0.248	Malic acid	0.184
Canola inclusion	0.291	Methylbutyrate inclusion	0.109
Carboxylic acid	0.636	Mimosa	0.310
Cerium chloride	0.078	Monensin	*<0.001*
Chestnut	*0.044*	Myristic acid	0.561
Chitosan	0.841	Nisin	0.205
Coconut inclusion	*0.005*	Nitrate	*<0.001*
Corn	0.183	Nitroethane	*0.045*
Cumin	0.069	Oregano	0.077
Cysteine	0.587	Polyethylene glycol	0.256
DDGS concentrate	*0.012*	Quebracho	0.109
DHA inclusion	0.372	Saifoin	*0.027*
Essential oil blend	0.172	Saponaria	0.177
Eugenol	*0.008*	*Sericea lespedeza* tannins	*0.008*
Fatty acid blend inclusion	0.101	Sorghum tannins	0.150
Fibrolytic enzyme	0.223	Soybean oil inclusion	0.749
Flaxseed inclusion	0.454	Stearic acid	0.719
Garlic	0.848	Sunflower inclusion	0.079
Glycerin	0.793	Tea saponin	0.096
Grape pomace	*0.050*	Triiodothyronine	0.695
Grass	0.676	Valonea	0.883
Hydrolysable tannins	*0.018*	Vitacogen	0.587
Iso-valerate inclusion	0.093	Yucca	0.172
Lasolocid	0.946		
Lauric	0.427		

Some data from Global Network project are included.

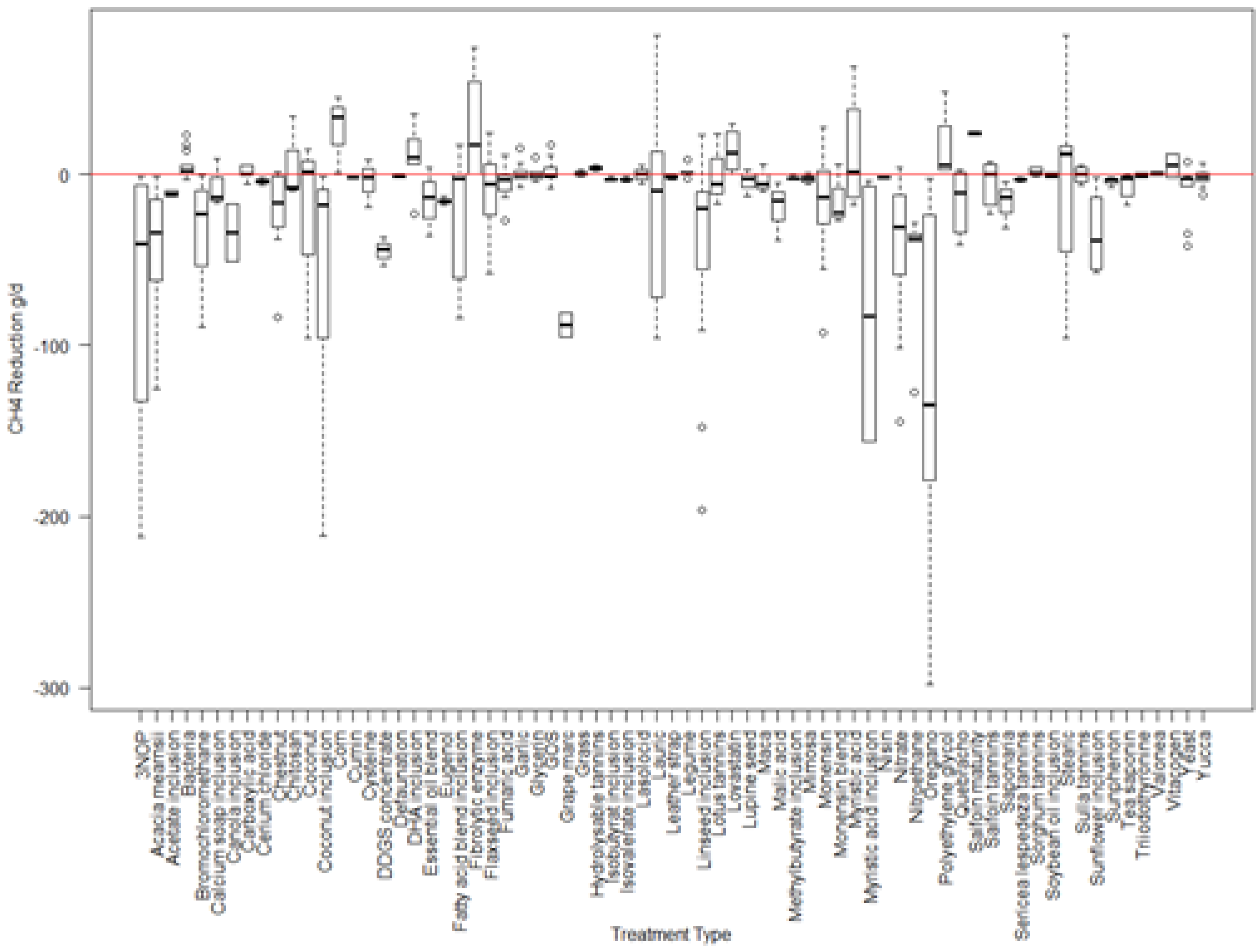
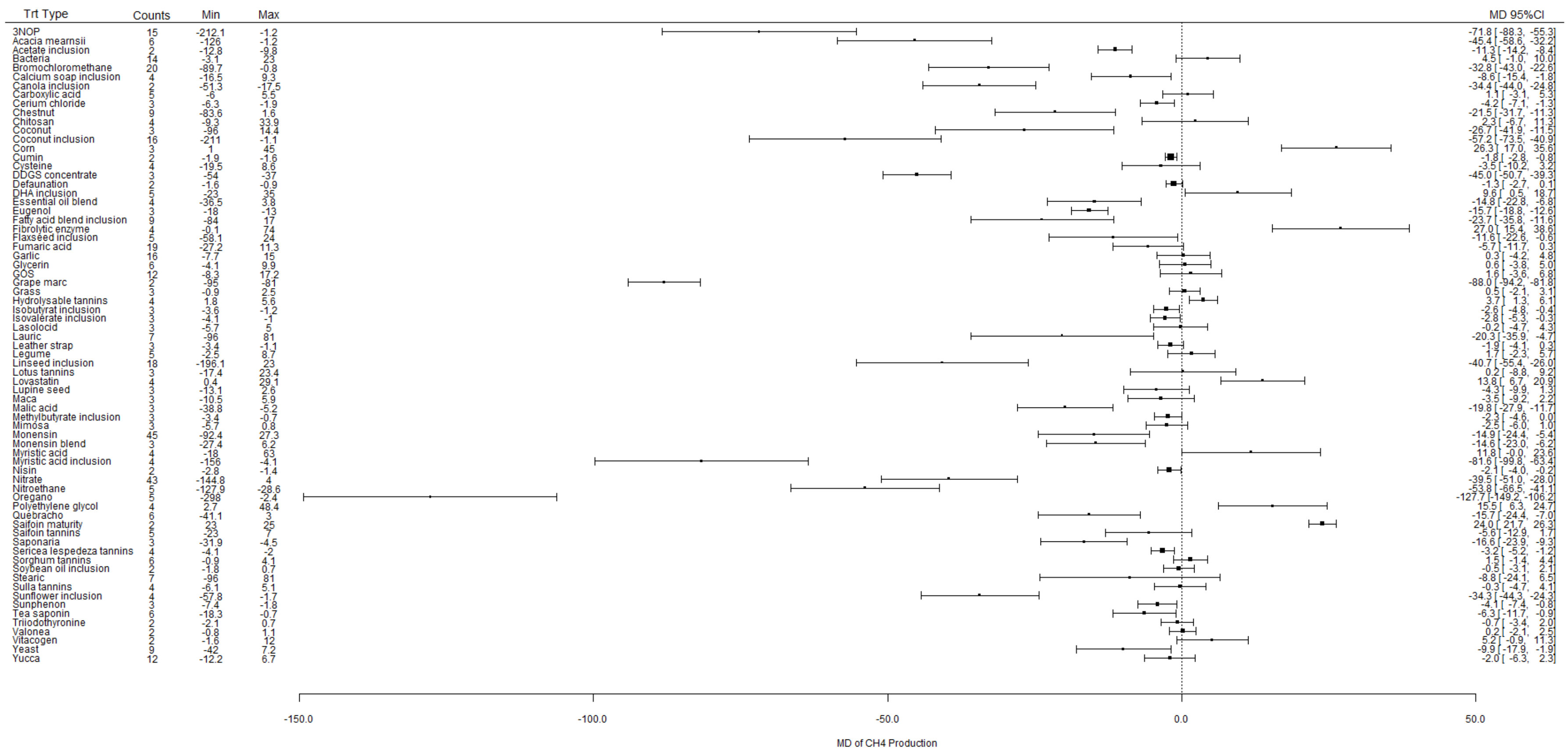


Figure 1. Boxplot of mean difference of CH_4 production. The horizontal line was the reference line of 0 g/d reduction. Some data from Global Network project are included.



Figures 2. Forest plot including the summary of valid treatment counts, lower and upper boundaries of mean difference for different feed additives with 95% credible interval. Some data from Global Network project are included.

Once the potential feed additives were identified, a secondary assessment was conducted to investigate their appropriateness for California livestock industry including cost, unintended negative consequences, availability and persistence in reducing emissions. The use of chestnut, coconut inclusion, DDGS concentrate, eugenol saifoin, fumaric acid and linseed appear to increase the cost of production as well as reduce productivity or pollutions swapping. For example increased use of DDGS may reduce methane but increases nitrogen loading, which may contribute to increased N_2O emissions. Therefore, these mitigation options were not considered further. The study by Appuhamy et al. (2013) showed that although monensin reduced methane production by about 6% in beef and 12% in dairy cattle, the effect was transient. After about 6 weeks of monensin supplementation the rumen microbes adapt to it and any benefit in reduction of methane emissions is lost. Therefore, monensin was not considered further.

Feed additives with some potential for mitigation

Secondary plant compounds

Tannins have shown promise for methane mitigation but not much work has been done in California conditions. There is a need for further investigation of the use of tannins, in particular grape pomace (or grape marc), in California, as the raw materials are easily available. UC Davis plans to conduct a trial on grape pomace. There are also feed additives that were not considered fully because of lack of studies. A feed additive based on citrus and garlic extracts, Mootral, has been studied in California. Roque et al. (2019a) showed that after 12 weeks of supplementation, Mootral reduced methane emissions by 23%. The trial was relatively short and involved 20 animals. A bigger trial with 45 beef cattle and longer period has been planned for summer 2020 at UC Davis. Following that, another trial with Mootral using dairy cattle is planned for fall 2020.

These studies will shed light to the effectiveness of Mootral under California conditions and need to be considered for use after the results are published. A research trial using Agolin has been conducted at UC Davis but results are not yet public and may be available in 2021.

Methanogenesis Inhibitors

Bromochloromethane in its pure form cannot be used as it is a banned substance under the Montreal Protocol. However, some seaweed species, particularly *Asparagopsis*, contain bromoform and bromochlormethane as active ingredients that has been shown to be effective *in vitro* (Machado et al., 2016). The first *in vivo* trial using *Asparagopsis* in cattle was conducted at UC Davis (Roque et al., 2019b) who reported up to 67% reduction in methane production in dairy cattle. The authors reported a decline in feed intake, particularly at the high level of inclusion, which might compromise milk production. Bromoform residue was not found in milk samples. A new paper was published during the final report-writing phase of this study. Kinley et al. (2020) reported that methane emissions in Brangus cattle declined 98% with inclusion of only 0.02% of *Asparagopsis taxiformis*. Additionally, they reported no reduction in feed intake or loss of productivity. Analysis of the meat from seaweed supplemented animals did not show any bromoform residue. Human consumption of high levels of bromoform could be hazardous, so the US EPA (2008) has set drinking water regulations on bromoform consumption to 80 mg/L. Another longer term study has been completed at UC Davis but results were not fully available as of the time of writing the report. Although there is no question regarding efficacy of *Asparagopsis*, some issues such as supply, cost and FDA approval remain to be solved, therefore, more research needs to be conducted to get it to market and being recommended for use in California.

Feed Additives with highest potential for mitigation

We found two feed additives that have been extensively studied (over 10 trials each). These are 3-nitrooxypropanol (3NOP), and nitrate. The rest of the section on feed additives will focus on these two. We updated a meta-analysis conducted for 3NOP and built a new meta-analysis for use of nitrate in beef and dairy cattle. We then proceeded to use a life-cycle assessment developed for California conditions (Naranjo et al. 2020) and assessed the ***net*** reduction expected if either 3NOP or nitrate were to be used. The life-cycle assessment was from cradle to farm gate so included emissions from feed production, the barn (i.e., animals, manure, electricity) and farm operations. There is a challenge of supplementing with nitrate currently due to toxicity risk. However, research is being conducted to add microbes that can help in detoxification (Latham et al., 2019) and a full analysis of nitrate use is provided below.

3-Nitrooxypropanol (3NOP)

A meta-analysis was conducted by Dijkstra et al. (2018) on effects of 3NOP. However, 4 additional papers were published that were not included in the meta-analysis. Therefore, we updated the previous meta-analysis by adding data from Martinez-Fernandez et al. (2018) (beef; 1 study), Vyas et al. (2018b) (beef; 2 studies), Kim et al. (2019) (beef; 4 studies), and Van et al. (2019) (dairy; 2 studies). The updated forest plots for Standardized Mean Difference of CH_4 production and yield are shown in Figures 3 and 4.

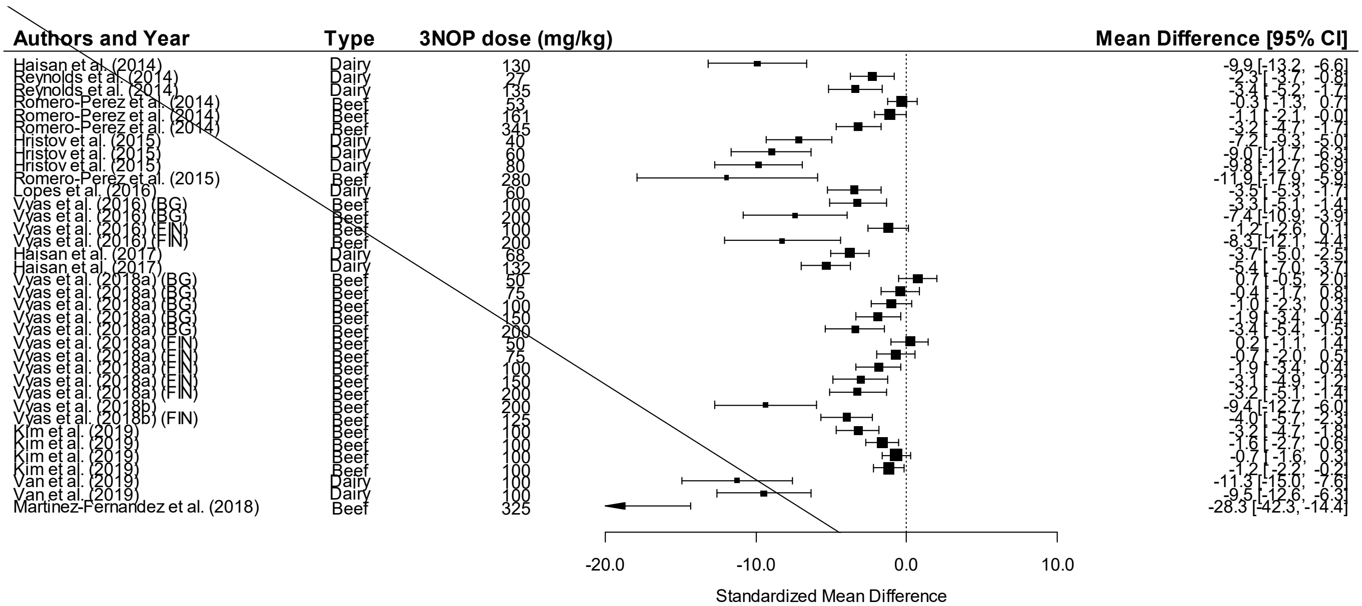


Figure 3. Forest plot showing 3-nitrooxypropanol (3NOP) dose (mg/kg of DM) and standardized mean difference (mean difference is calculated as NOP treatment mean − control treatment mean) in CH_4 production (g/d) for beef and dairy cattle studies.

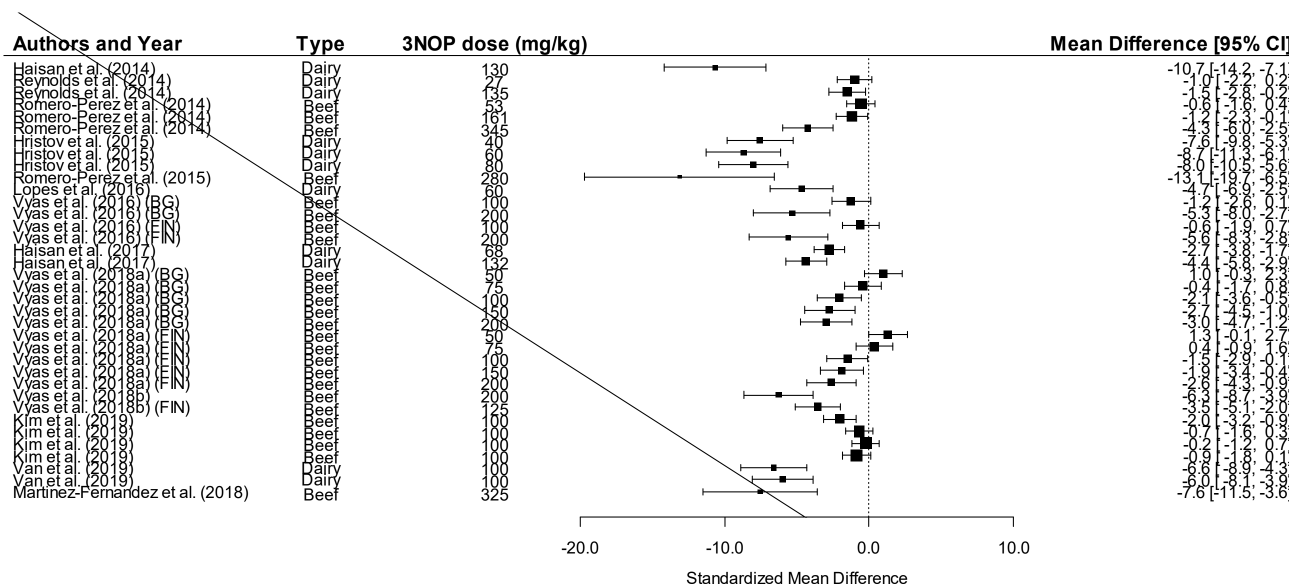


Figure 4. Forest plot showing 3-nitrooxypropanol (3NOP) dose (mg/kg of DM) and standardized mean difference (mean difference is calculated as NOP treatment mean − control treatment mean) in CH_4 yield (g/kg of DMI) for beef and dairy cattle studies.

The data was checked if it fits a normal distribution function and for outliers. A quantile-quantile plot (Q-Q plot), showed that the data was normally distributed (Figure 5), therefore, no outliers were removed before conducting the meta-analysis.

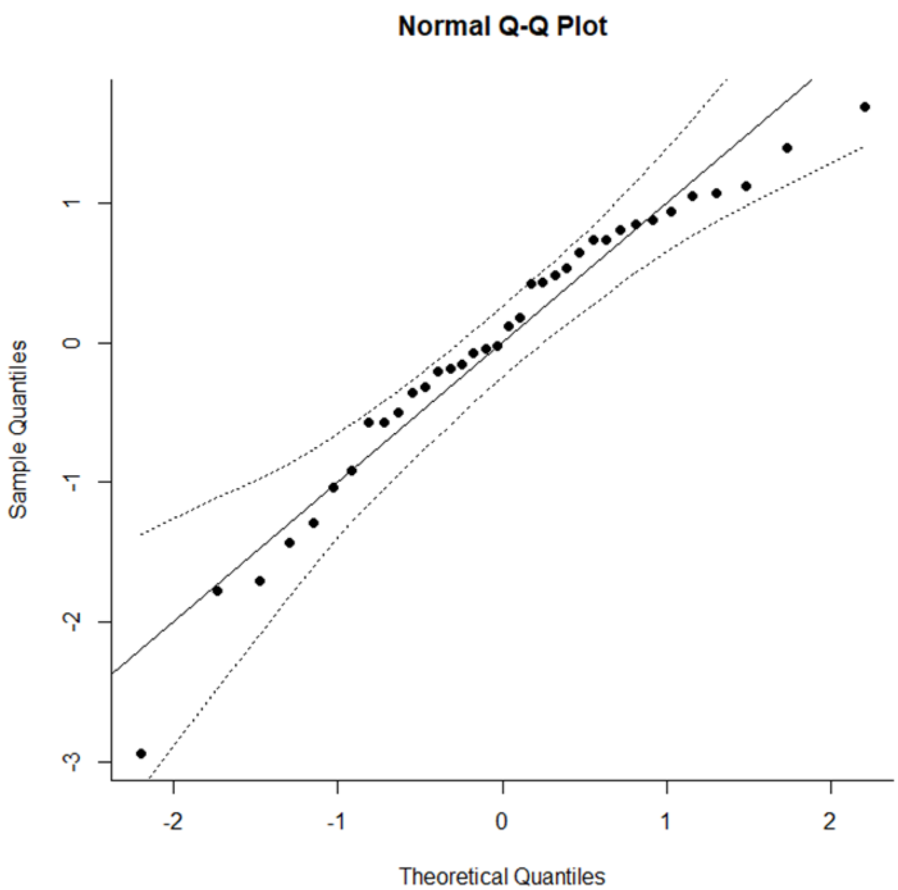


Figure 5. A quantile-quantile plot (Q-Q plot) of the data.

The results of the mixed-effect models for CH_4 production and yield was similar to the previous study which indicated effectiveness of 3NOP at mitigating CH_4 emissions (Table 2). As expected, the effect was positively associated with dose, and negatively associated with dietary fiber content. Moreover, NOP had stronger anti-methanogenic effects in dairy cattle than in beef cattle. The mean value of NOP dose was 127 mg/kg of DM which slightly increased comparing to the 123 mg/kg of DM in previous analysis. The overall mitigating effect of 3NOP was 32% at 127 mg/kg inclusion level. In dairy cattle specifically the impact was 41% reduction while in beef cattle it was 22.4% (Table 2).

Table 2. Estimates of overall 3-nitrooxypropanol (3NOP) effect size and of explanatory variables from random- and mixed-effect models for relative mean difference (MD, %) in CH_4 production (g/d) and yield (g/kg of DMI)

Variable and model	CH4 production				CH4 yield			
	Mean	SE	P-value	τ2	Mean	SE	P-value	τ2
Random-effect model								
Overall NOP effect size	-32.0	4.46	<0.001	210	-29.6	4.58	<0.001	397
Mixed-effect model, 1 explanatory variable								
Overall NOP effect size	-30.4	4.16	<0.001	331	-27.8	4.19	<0.001	305
NOP dose (mg/kg of DM)	-0.114	0.0563	0.0996		-0.128	0.0464	0.0401	
Final mixed-effect model-I								
Dairy cattle	-41.5	4.82	<0.001	128	-39.8	5.17	<0.001	162
Beef cattle	-22.8	3.68	<0.001		-19.3	3.78	0.0020	
NOP dose (mg/kg of DM)	-0.260	0.0538	0.0031		-0.265	0.0618	0.0054	
NDF content (g/kg of DM)	0.129	0.0282	0.0040		0.109	0.0310	0.0131	
If removed the 2 studies in Kim et al. (2019), final model selection did not change								
Random-effect model								
Overall NOP effect size	-32.0	4.45	<0.001	210	-29.6	4.58	<0.001	397
Mixed-effect model, 1 explanatory variable								
Overall NOP effect size	-30.6	4.17	<0.001	331	-28.0	4.19	<0.001	305
NOP dose (mg/kg of DM)	-0.114	0.0563	0.0998		-0.128	0.0464	0.0401	
Final mixed-effect model-I								
Dairy cattle	-41.0	4.83	<0.001	129	-39.5	5.20	<0.001	163
Beef cattle	-22.4	3.53	<0.001		-19.1	3.66	0.0018	
NOP dose (mg/kg of DM)	-0.258	0.0534	0.0031		-0.262	0.0613	0.0055	
NDF content (g/kg of DM)	0.127	0.0294	0.0053		0.106	0.0324	0.0178	

Nitrates

Nitrate (NO_3^-) is a strong inorganic anion and acts as an alternative hydrogen sink in rumen to potentially compete with methanogens for hydrogen utilization. Dietary nitrate is firstly reduced to nitrite (NO_2^-; $NO_3^- + H_2 \rightarrow NO_2^- + H_2O$) and then to ammonia ($NH_4^+$; $NO_2^- + 3H_2 + 2H^+ \rightarrow NH_4^+ + 2H_2O$) which is energetically more favorable than the reduction of CO_2 to CH_4 ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) due to a higher Gibbs energy change (Villar et al., 2020). Thus, nitrate reduction is highly competitive compared with methanogenesis that leads a redirection of H flow away from CO_2 reduction, and thereby reduces enteric CH_4 production (Olijhoek et al., 2016).

Several *in vivo* studies have investigated the effects of nitrate as a CH_4 mitigation strategy in different types of ruminants such as beef steers (Hulshof et al., 2012; Troy et al., 2015; Alemu et al., 2019), dairy cows (Veneman et al., 2015; Klop et al., 2016; Meller et al., 2019) , sheep (Sar et al., 2004; van Zijderveld et al., 2010,), and goats (Zhang et al., 2019). However, the results of the trials on effectiveness of nitrate mitigation on CH_4 emissions for ruminants have been inconsistent with large variability. The studies were conducted under various dietary regimen and nitrate doses so some of the differences may be explained by dietary or other variables. For example, Guyader et al. (2015a) reported that CH_4 yield in nitrate treatment group was reduced 22% in nonlactating Holstein cows and Lee et al. (2015) showed that CH_4-mitigating effect of nitrate for beef heifers were associated with nitrate dose, and the reduction rates varied from 3.3 to 20.8%. However, van Wyngaard et al. (2019) did not find a significant effect on mitigating CH_4 emissions when dietary nitrate was fed to dairy cows grazing perennial ryegrass.

The objective of this study was to collate data on nitrate supplementation for CH_4 mitigation and quantitatively evaluate the effects of dietary nitrate for enteric CH_4 production and yield. Nitrate dose, nutrient composition of diet, dry matter intake, and cattle type may potentially explain a large proportion of the between-study variability in CH_4 mitigation effect of nitrate (Lee and Beauchemin, 2014; Dijkstra et al., 2018). Therefore, this study quantitatively analyzes explanatory variables to account for the heterogeneity observed in emission reduction due to nitrate in diet using a meta-analysis approach.

Materials and methods

Literature search was conducted using several sources including the Web of Science (Thomson Reuters Science, New York, NY), Elsevier (Elsevier, Amsterdam, the Netherlands) and Google Scholar online databases with all possible combinations of the keywords "feed additives", "nitrate", "methane" (including all variants of "CH_4" and "greenhouse gas"), "cattle" (including all variants of "dairy", "beef", "steer", "cows" and "ruminants"). The period of the study covered from 1970 to 2019. The search resulted in 45 references related to the effects of nitrate on enteric CH_4 production in cattle. All the references were scrutinized by reading the abstracts, experimental design, and results of each reference carefully. To be included in the database, the studies were required to meet the following criteria: (i) a control group treatment group that did not receive nitrate; (ii) to be conducted *in vivo* using cattle; (iii) reported CH_4 production with standard deviation, standard error or other relative data that can be used to calculate the standard error (e.g. least significant difference); (iv) described other required variables (e.g. nutritional composition) or provided enough information to estimate the variables. Of the 45 references, two were general summary papers and three articles had only abstracts available so these were excluded from the dataset. Three papers were removed because they investigated the mitigation effect on CH_4 of a mixture of nitrate and other feed additives. Five papers did not report CH_4 emissions and another five did not provide diets or dietary information useful in calculating them, therefore were not included in the database. Data from 27 articles met the criteria, however, another three articles were rejected because data were duplicates of references already included in the database. The remaining 24 articles containing 57 treatment means were selected for the final database. Of those 36 treatments were related to beef cattle (Hulshof et al., 2012; Newbold et al., 2014; Lee et al., 2015; Troy et al., 2015; Lee et al., 2017a, b; Capelari, 2018; Duthie et al., 2018; Henry et al., 2018; Tomkins et al., 2018; Alemu et al., 2019; Granja-Salcedo et al., 2019; Rebelo et al., 2019) and 21 treatments to dairy cattle (van Zijderveld et al., 2011; Guyader et al., 2015a, b; Veneman et al., 2015; Klop et al., 2016; van Wyngaard et al., 2018; Meller et al., 2019; van Wyngaard et al., 2019).

The primary response variables included the means of CH_4 production and yield in control and nitrate treatment groups. Factors having a potential to explain the variability in nitrate effect on CH_4 emissions were selected and considered in the meta-analysis. Methane production was generally reported in grams per day and CH_4 yield in grams per kilogram of DMI. If the values were reported in liters or moles per day, they were converted to grams per day assuming a volume of 22.4 L and molar weight of 16.0 g. If only one of the CH_4 production and CH_4 yield was given, the other variable was calculated as CH_4 yield = CH_4 production/DMI.

General meta-regression methods require the independency of effect sizes (i.e., the quantitative measure of the difference in magnitude in methane emission between control and treatment). However, multiple nitrate treatment groups may share a same control treatment group in some of the studies used in our database. To deal with the unknown correlations among these non-independent effect sizes, a robust variance estimation (RVE) method (Tipton, 2015) was used to conduct the meta-analysis. Studies selected in the meta-analysis were not identical in the methods and sample characteristics which may introduce variance of the true effect sizes, therefore, RVE random-effects and RVE mixed-effects models were fitted to estimate between-study variability (heterogeneity) that was assumed to be purely random (Tanner-Smith et al., 2016; Dijkstra et al., 2018). The RVE random-effects model was written as

$$y_{ij} = \beta_0 + \mu_j + e_{ij},$$

where for i =1,…, k_j, j = 1,…, m, y_{ij} is the ith effect size of jth study, β_0 is the average true effect, μ_j is the random effect at study level where $\mu_j \sim$ N (0, τ^2) and τ^2 is the between-study variance component, and e_{ij} is the residual for ith effect size in the jth study where $e_{ij} \sim$ N (0, s_i^2) and s_i^2 is the error variance component. The heterogeneity (I^2) is defined as the ratio of between-study variance (τ^2) to the total variability ($s_i^2 + \tau^2$) and an I^2 value greater than 0.5 indicates significant heterogeneity (Dijkstra et al., 2018). To examine effect size moderators and reduce heterogeneity, the RVE random-effects models can be extended to RVE mixed-effects models which include variables with the potential to account for some of the observed variability. The RVE mixed-effects model was written as

$$y_{ij} = \beta_0 + \mu_j + \mathbf{X}_{ij}\boldsymbol{\beta} + e_{ij},$$

where β_0, μ_j, and e_{ij} are as defined above, $\boldsymbol{\beta}$ = (β_1,… β_p) is a vector of unknown regression coefficients based on weighted least-squares estimates, and $\mathbf{X}ij$ is a vector of continuous or binary explanatory variables. The inverse variance weights of “correlated effects” used in RVE models were estimated following a method provided by Hedges et al. (2010):

$$\mathrm{w}_{ij} = \frac{1}{k_j(\upsilon_{.j} + \tau^2)}$$

where w_{ij} is the ith inverse variance weight in jth study, k_j is the number of effect sizes for each study j, $\upsilon_{.j}$ is the mean of within-study sampling variances (υ_{ij}) for the k_j effect sizes in jth study, and τ^2 is the between-study variance component as defined previously which describes the residual of heterogeneity that is not explained by the involved variables.

The dry matter intake (DMI), body weight (BW), roughage proportion in the diet, dietary crude protein (CP), and neutral detergent fiber (NDF), and nitrate dose were selected as potential continuous explanatory variables. Types of cattle (dairy or beef) were used as category variables. Therefore, the vector $\boldsymbol{\beta}$ can be explained as the differences in true effect sizes according to each unit changing in the continuous variables or between the two cattle types. The RVE model was first fitted with each individual variable, and two or more variables were included following a stepwise method until all explanatory variables were involved to conduct full mixed-effect models (Dijkstra et al., 2018). Only the variables showing significant effects ($P < 0.10$) were retained until the final model was selected. Multi-collinearity was investigated to examine the correlations among variables and highly correlated variables ($|r| > 0.50$) were not analyzed in the same model such as DMI and CP ($|r| = 0.59$), and CP and NDF ($|r| = 0.51$). All explanatory variables (except for cattle types) were first centered on their means. Potential variables such as gross energy (GE) content, ash content, fat content, and organic matter (OM) digestibility were also considered in data collection, however, due to the lack of information in most of the publications, they were not included in this analysis.

To prepare for the meta-analysis, effect size estimates of mean difference (MD) and standardized mean difference (SMD) were used to measure the continuous response variables of CH_4 production and yield. The MD was calculated as nitrate treatment mean minus control treatment mean and each study was weighted by its corresponding sample variation (Viechtbauer, 2010). The SMD was expressed as dividing MD by the pooled standard deviation of the two group (SMD = MD/pooled standard deviation of the 2 groups) and used to construct forest plots of response variables. The relative mean difference (RMD; RMD = MD/control treatment mean × 100%), which was a dimensionless variable, was calculated for further analyses to eliminate the large variations and different measuring scales of DMI and CH_4 production from study to study.

All statistical analyses were carried out using various packages in R (version 3.6.1, R Foundation for Statistical Computing, Vienna, Austria). The "cor" function in R (version 3.6.1) was used to test the correlation between explanatory variables. The "escalc" and "robu" functions provided by "metafor" (version 2.1-0) and "robumeta" (version 2.1) packages in R were used to calculate effect sizes (MD and SMD) and conduct RVE models, respectively.

Results and Discussion

Meta-analysis is a statistical methodology that combines quantitative findings from various studies for the main purpose of synthesizing the evidence based on the available sources (Schwarzer et al., 2015). The meta-analysis conducted in this paper aimed to evaluate the effects of nitrate as a feed additive to reduce CH_4 production and yield in dairy and beef cattle. A summary statistic of feed intake, nutrient compositions of the experimental diet, nitrate supplement, and CH_4 production is given in Table 3. The daily DMI and CH_4 production of dairy cows (16.2 ± 2.86 kg/d; 286 ± 52.1 kg/d, respectively) were greater than beef steers (9.5 ± 4.20 kg/d; 137 ± 47.2 kg/d), while the averages of supplemented nitrate dose were not significantly different between dairy (18 g/kg of DM) and beef cattle (17 g/kg of DM). On average, the effects of nitrate resulted in greater RMD in CH_4 production and yield for dairy cows (-16.7 ± 7.64%; -15.4 ± 7.66%) than those for beef steers (-12.3 ± 10.22%; 9.0 ± 11.15%). Forest plots generated with SMD for CH_4 production (Figure 6) and CH_4 yield (Figure 7) showed consistent anti-methanogenic effects in most of the studies included in this analysis. However, effect sizes were variable across studies.

At an average nitrate dose of 18 g/kg of DM, the overall CH_4 production ($P < 0.001$) and CH_4 yield ($P < 0.001$) were reduced by 14.4 ± 1.21% in dairy and 11.4 ± 1.40%, in beef cattle according to the random-effect RVE models (Table 4). Several other feed additives have also shown to reduce methane emissions but mostly at a lower effectiveness. For example, Appuhamy et al. (2013) reported monensin reduced CH_4 production by 5.6% for dairy cows and 4.6% for beef steers. Eugène et al. (2008) investigated lipid supplementation and reported it reduced CH_4 production by 9.0% in lactating dairy cows. Van Zijderveld et al. (2011) observed a 10% decrease in CH_4 emissions by supplementing mixed additives of lauric acid, myristic acid, and linseed oil in dairy cattle. However, 3NOP showed stronger antimethanogenic effects with 39% and 22% reduction level of CH_4 production in dairy and beef cattle, respectively (Dijkstra et al., 2018). Similarly, Roque et al. (2019a) reported Mootral reduced CH_4 production 23% after 12 weeks of supplementation in beef cattle.

Table 3. Summary statistics of dietary composition, feed intake, animal characteristic, and methane emission of the database.

Item	Dairy					Beef				
	Mean	Median	SD[3]	Min	Max	Mean	Median	SD[3]	Min	Max
DMI (kg/d)	16.2	17.6	2.86	10.2	19.7	9.5	8.3	4.20	6.1	22.9
Roughage proportion (% of diet DM)	61	60	10.6	50	78	62	65	21.6	10	100
NDF (g/kg of DM)	356	352	67.2	100	426	372	362	120.3	227	680
CP (g/kg of DM)	149	156	21.3	88	175	129	134	22.3	49	150
BW (kg)	466	533	187.7	117	658	430	337	147.2	283	698
Nitrate dose (g/kg DM)	18	21	4.7	5	23	17	19	5.4	5	27
CH_4 production (g/d)	286	300	52.1	175	405	137	140	47.2	71	243
MD[1] of CH_4 production (g/d)	-57	-59	26.9	-100	5	-19	-18	15.1	-43	31
RMD[2] of CH_4 production (% of control)	-16.7	-17.0	7.64	-29.8	1.3	-12.3	-11.4	10.22	-32.0	22.0
CH_4 yield (g/kg of DMI)	17.9	17.4	2.17	14.5	24.3	17.2	17.9	4.87	8.8	27.6
MD of CH_4 yield (g/kg of DMI)	-3.3	-3.3	1.86	-6.8	1.1	-1.7	-1.7	1.97	-5.7	3.3
RMD of CH_4 yield (% of control)	-15.4	-14.9	7.66	-27.6	4.7	-9.0	-9.5	11.15	-29.4	19.3

[1]MD (Mean difference) = treatment mean - control mean.
[2]RMD (Relative mean difference) = (MD/control mean) × 100%.
[3]SD = standard deviation of mean

Table 4. Estimates of overall nitrate effect from random-effect model, and of explanatory variables from mix-effect models for relative mean difference (RMD) in CH_4 production (g/d) and yield (g/kg of DMI).

Variable[1] and model	CH_4 production				CH_4 yield			
	Mean	SE	*P*-value	τ^2	Mean	SE	*P*-value	τ^2
Random-effect model								
Overall effect size	-14.4	1.21	<0.001	53.0	-11.4	1.40	<0.001	53.6
Mixed-effect model, 1 explanatory variable[2]								
Model I: Overall effect size	-14.2	1.05	<0.001	29.4	-11.5	1.30	<0.001	46.2
Nitrate dose (g/kg of DM)	-0.932	0.195	<0.001		-0.776	0.235	0.004	
Model II: Dairy cattle					-15.4	1.71	<0.001	52.6
Beef cattle					-9.03	1.90	<0.001	
Mixed-effect model, 2 explanatory variables[3]								
Model I: Dairy cattle	-14.1[4]	1.79	<0.001	29.0	-14.9[4]	1.36	<0.001	47.7
Beef cattle	-14.2	1.32	<0.001		-9.40	1.88	<0.001	
Nitrate dose (g/kg of DM)	-0.933	0.203	<0.001		-0.720	0.246	0.009	
Model II: Dairy cattle					-15.7[4]	1.50	<0.001	54.5
Beef cattle					-9.16	1.86	<0.001	
NDF content (g/kg of DM)					-0.0321	0.0164	0.083	
Model III: Nitrate dose (g/kg of DM)					-0.936	0.429	0.040	199
NDF content (g/kg of DM)					-0.0366	0.0161	0.042	
Final mixed-effect model								
Model I: Dairy cattle	-14.3[4]	1.49	<0.001	27.2	-15.2[4]	1.05	<0.001	30.7
Beef cattle	-14.0	1.35	<0.001		-9.82	1.66	<0.001	
Nitrate dose (g/kg of DM)	-1.01	0.232	<0.001		-0.967	0.229	<0.001	
NDF content (g/kg of DM)	-0.0214	0.0135	0.144		-0.0471	0.0129	0.004	

[1]The explanatory variables centered on their means (except cattle type variable): BW = 443 kg; CP content = 137 g/kg of DM; NDF content = 366 g/kg of DM; roughage proportion = 61%; DMI = 12.0 kg/d; nitrate dose = 18 g/kg of DM.

[2] Mixed-effect models with 1 explanatory variable had no significant effect on CH_4 production or CH_4 yield were not listed. Variables included: BW ($P = 0.905$), NDF ($P = 0.500$), CP ($P = 0.407$), roughage proportion ($P = 0.802$), DMI ($P = 0.994$), and cattle type ($P = 0.432$) for CH_4 production; BW ($P = 0.765$), NDF ($P = 0.112$), CP ($P = 0.537$), roughage proportion ($P = 0.342$), and DMI ($P = 0.417$) for CH_4 yield.

[3] Mixed-effect models with 2 and more explanatory variables that had no significant effect on CH_4 production or CH_4 yield were not retained.

[4] Cattle type effects for CH_4 production were not significant ($P > 0.50$); for CH_4 yield were significant ($P < 0.05$).

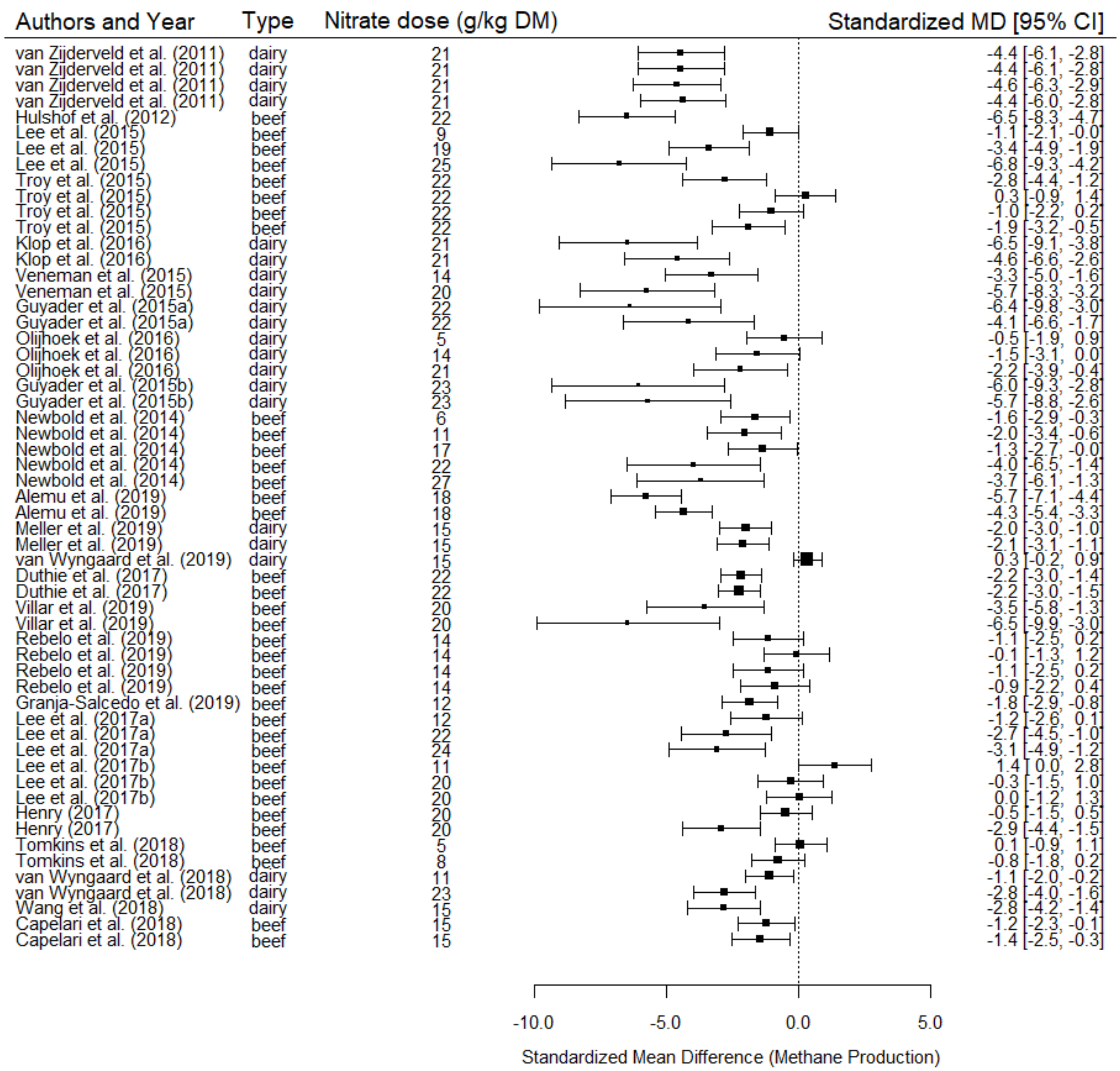


Figure 6. Forest plot showing nitrate dose (g/kg of DM) and standardized mean difference in CH_4 production (g/d) and its 95% confidence interval (CI) for beef and dairy cattle from selected studies. The dotted line represents a reference of 0 standardized mean difference. The black squares represent the power of its corresponding studies (Note: A larger box indicates a greater sample size and a smaller CI).

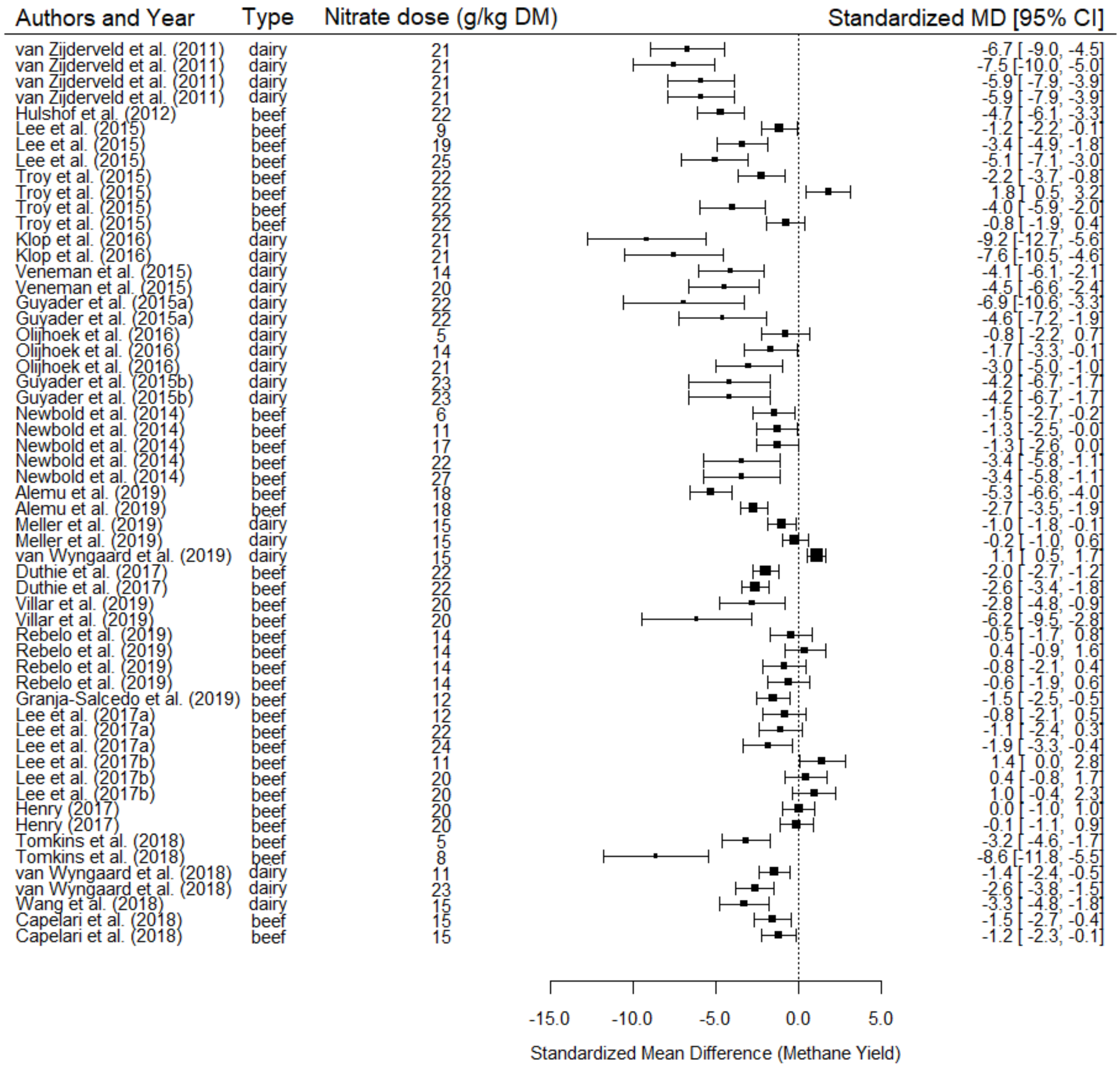


Figure 7. Forest plot showing nitrate dose (g/kg of DM) and standardized mean difference in CH_4 yield (g/kg of DMI) and its 95% confidence interval (CI) for beef and dairy cattle from selected studies. The dotted line represents a reference of 0 standardized mean difference. The black squares represent the power of its corresponding studies (Note: A larger box indicates a greater sample size and a smaller CI).

The RVE random-effect models showed that a large proportion of the total variability of nitrate effects on CH_4 production (I^2 = 69.9%) and CH_4 yield (I^2 = 99.7%) were attributed to heterogeneity. Potential explanatory variables were individually included to conduct mixed-effect RVE models to further understanding and improve the random-effect models (Table 4). The size of CH_4 production reduction was positively associated with nitrate dose ($P < 0.001$). A 10 g/kg of DM increase in nitrate dose from its mean (18 g/kg of DM), enhanced the nitrate anti-methanogenic effect of CH_4 production by 9.32 ± 1.95%. However, for RMD in CH_4 production, the categorical variable cattle type ($P = 0.432$), and continuous variables BW ($P = 0.905$), NDF content ($P = 0.500$), CP content ($P = 0.407$), roughage proportion of diet ($P = 0.802$), and DMI ($P = 0.994$) were not significant. For RMD in CH_4 yield, BW ($P = 0.765$), NDF content ($P = 0.112$), CP content ($P = 0.537$), roughage proportion of diet ($P = 0.342$), and DMI ($P = 0.417$) were not significant. But, the categorical variable cattle type ($P = 0.017$) and nitrate dose ($P = 0.004$) were significant. A 10 g/kg of DM increase in nitrate dose from its mean (18 g/kg of DM) resulted in 7.76 ± 2.35% decline in CH_4 yield (Table 4, Model I). A 10 g/kg of DM increasing in nitrate dose from its mean (18 g/kg of DM) resulted in 7.76 ± 2.35% decline of CH_4 yield (Model I). The results agree with Lee and Beauchemin (2014) in which they reported a linear reduction in CH_4 yield with increasing levels of nitrate dose. Nitrate mitigation effect on CH_4 yield in dairy and beef cattle were -15.4 ± 1.71% and -9.03 ± 1.90%, respectively, that were significantly different from each other according to Model II (Table 4). This indicates that nitrate shows a stronger impact on mitigating CH_4 yield for dairy cattle, and a higher nitrate dose is required for beef cattle to obtain the same effectiveness at reducing CH_4 yield compared that for dairy cattle. The heterogeneity was reduced by including the individual explanatory variable for both CH_4 production (τ^2 = 53.0 vs. 29.4) and CH_4 yield (τ^2 = 53.6 vs. 46.2 for Model I or 52.6 for Model II).

Adjusting the RVE mixed-effect model to use two explanatory variables, cattle type ($P <$ 0.001) and nitrate dose ($P < 0.001$; $P = 0.009$) were significantly associated with nitrate effect on CH_4 production and yield (Model I). A 10 g/kg of DM increase in nitrate dose enhanced the nitrate effect on CH_4 production by 9.33 ± 2.03% from the average of 14.1 ± 1.79% for dairy cows, and 14.2 ± 1.32% for beef steers. Similar increase in nitrate dose enhanced the nitrate effect on CH_4 yield by 7.20 ± 2.46% from the average of 14.9 ± 1.36% for dairy cows, and 9.40 ± 1.88% for beef steers. The mixed-effect model conducted with cattle type and nitrate dose slightly reduced the heterogeneity for CH_4 production (τ^2 = 29.4 vs. 29.0), however, the heterogeneity for CH_4 yield was not improved by the model (τ^2 = 46.2 vs. 47.7). When the model was adjusted for NDF content instead of nitrate dose (Model II), CH_4 yield tended to decline ($P = 0.083$) by 0.321 ± 0.164% for every 10 g/kg of DM increase in NDF content from the average in dairy (-15.7 ± 1.50%) and beef (-9.16 ± 1.86%) cattle. Although, nitrate dose ($P = 0.040$) and NDF content ($P = 0.041$) were significantly related to nitrate effect on CH_4 yield in Model III, the heterogeneity jumped from 47.7 (Model I) or 54.5 (Model II) to 199 (Model III) indicating the importance of including cattle type in the model.

The final mixed-effect models for RMD in CH_4 emissions (Table 4) included cattle type, nitrate dose and dietary NDF content. The τ^2 decreased from the random-effect model to a mixed-effect model with 1 and 2 explanatory variables, and further decreased to the final mixed-effect model with 3 explanatory variables (CH_4 production: τ^2 = 27.2 vs. 53.0; CH_4 yield: τ^2 = 30.7 vs. 53.6) but not with 2 explanatory variables for CH_4 yield (Table 4). When adjusted for the effects of nitrate dose and dietary NDF content, the anti-methanogenic effect of nitrate was similar in beef cattle (-14.0 ± 1.35%; P <0.001) compared to dairy cattle (−14.3 ± 1.49%; P <0.001) for CH_4 production. However, for CH_4 yield, with nitrate dose centered on its mean (18 g/kg of DM), and the mean NDF content of 366 g/kg of DM, the anti-methanogenic effect of nitrate was stronger in dairy cows (-15.2 ± 1.50%; P <0.001) compared to beef cattle (-9.82 ± 1.66%; P <0.001). The greater efficacy in dairy cattle may be related to the differences in the levels of feed intake (dairy: 16.2 kg/d, beef: 9.5 kg/d; Table 3. A similar difference in cattle type on efficacy of 3NOP was reported (Djikstra et al., 2018). The authors suggested that higher feed intake levels increase rumen concentrations of fermentation products, including volatile fatty acids and hydrogen and sinks of hydrogen in the rumen may be affected by hydrogen partial pressure. This will likely result in greater alternative hydrogen sinks for rumen methanogenesis. The efficacy of nitrate-N utilization may be improved, and the potential of nitrate inhibitory effect is enhanced through more completed nitrate reductions. After adjusting for cattle type and dietary NDF content in final mixed-effect models, the nitrate-induced CH_4 mitigation was 10.1 ± 2.32% (CH_4 production, $P < 0.001$) and 9.67 ± 2.29% (CH_4 yield, $P < 0.001$) per 10 g/kg of DM increase in nitrate dose from its mean (18 g/kg of DM; Table 4), which is slightly higher than the effect of nitrate dose observed in the individual and two explanatory variables mixed-effect models. In our analysis, an increase in dietary NDF content did not significantly affect the efficacy of nitrate in reducing CH_4 production ($P = 0.144$) but slightly increased ($P = 0.004$) the nitrate effect on CH_4 yield (Table 4). A 10 g/kg of DM increase in dietary NDF content from its mean (366 g/kg of DM) increased the nitrate effect on CH_4 yield by only 0.471 ± 0.129%.

ADDITIVES TARGETING MANURE METHANE EMISSIONS

Direct emissions of CH_4 and N_2O from livestock manure vary by manure treatment and storage methods. Both CH_4 and N_2O emissions can be mitigated either by reducing them during manure storage or maximizing CH_4 production and capturing the gas to produce biogas energy (USEPA, 2017). The greenhouse gas and odor emitted from manure and slurry could be directly or indirectly reduced through different technologies such as solids separation (Martinez et al., 2003; Owusu-Twum et al., 2017), dietary management strategies (Hristov et al., 2013; Lund et al., 2014; Troy et al., 2015), anaerobic digestion (Clemens et al., 2006), manure coverage (Misselbrook et al., 2016), and use of manure additives (Chen et al., 2018; Mao et al., 2018; Owusu-Twum et al., 2017; Wheeler et al., 2010; Yamulki, 2006).

Manure additives or amendments can be defined as substances that can be used to alleviate gaseous emissions associated with livestock manure handling and management. The application of manure additives is regarded as a practical and economical treatment method compared to alternative technology such as solids separation and biogas production (McCrory and Hobbs, 2001). Various types of additives have been applied on-farm and are reported in the literature over the last few decades, however, the effectiveness and performance for mitigating gas emissions of specific additives are not consistent, especially for the effects on CH_4 emissions. For example, Liu et al. (2017) and Vandecasteele et al. (2016) investigated the use of biochar and reported that it enhanced the organic matter degradation and reduced CH_4 emissions, however, Sanchez-Garcia et al. (2015) reported that there was no significant evidence showing the relevant impact of biochar on CH_4 emissions. A meta-analysis synthesizes the evidence from many available sources and combines and compares the treatment effects of individual studies by statistical methods (Dijkstra et al., 2018). The objective of this review was to investigate and quantitatively evaluate the effects of different types of manure additives on mitigating CH_4 emissions in livestock based on published literature data.

Data collection and selection

The main purposes for adding manure additives include directly reducing gas emission during storage and composting, and enhancing the gas emission to generate biogas. Literature searches of the Web of Science (Thomson Reuters Science, New York, NY) and Google Scholar online databases were conducted using the combination of search terms "manure additives", "methane" or "CH_4", "greenhouse gas", "reduce" or "reduction", "mitigate" or "mitigation", "amend" or "amendment". The covered period was from 2000 to 2019. A total of 42 papers were collected after the initial searching. All the references were carefully scrutinized by reading the abstracts, experimental design, and results. For inclusion in the database, the studies were required to include: (i) a control group, (ii) the CH_4 emissions reported with mean, standard deviation or standard error, and sample size and (iii) at least one type of additive was added directly into the manure for CH_4 emission reduction purposes. Studies related to increasing biogas generation by adding manure additives were not included because the objective of the manure additives was not to reduce emissions. There were 27 references remaining in the database after filtering by the criteria mentioned above. The manure additives were firstly categorized into general groups based on their function which were "acidification", "adsorbent", "biochar", "biological material", "C/N content", "disinfection", "essential oils", "humate", "microbial digestion", "oxidizing agent", "physical material", "straw", and for those that did not fit the above categories were put into "other chemicals" (Agyarko-Mintah et al., 2017; Berg et al., 2006; Chen et al., 2018; Chen et al., 2017; Chowdhury et al., 2014a; Chowdhury et al., 2014b; Hao et al., 2005; He et al., 2019; Jia et al., 2016; Liu et al., 2017; Luo et al., 2013; Mao et al., 2018; Martinez et al., 2003; Misselbrook et al., 2016; Owusu-Twum et al., 2017; Petersen et al., 2012; Regueiro et al., 2016; Samer et al., 2014; Shah & Kolar, 2012; Sommer & Moller, 2000; Sonoki et al., 2011; Vandecasteele et al., 2016; Wang et al., 2014; Wang et al., 2018; Wheeler et al., 2010; Yamulki, 2006; Zhang et al., 2017). A full list of studies investigated is given in Appendix 2. Statistical analysis and meta-analysis were both conducted based on the first level of classification due to the insufficient database for each type of second classified level. The manure came from various species of animals and were not sorted by species to obtain enough sample sizes for different manure additives.

Statistical analysis

All statistical analyses were conducted using R statistical software (version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria). A statistical summary of the whole dataset was conducted based on the calculated CH_4 reduction rate using the dplyr package in R. Each of the manure additive groups were subjected to significance test ($\alpha = 0.05$) to determine if they were effective in reducing manure CH_4 emissions. The manure additives that significantly reduce emissions were then included in further meta-analysis.

The response variable was the mean CH_4 production. However, different papers reported the CH_4 production in various units and different scales, such as, "g/m^2 per d", "g/m3", "g/d", "g/kg total solid", and "g/t fresh weight". The CH_4 emissions were recorded either as daily average or in cumulative total through the experimental period. To make the emission data comparable and eliminate bias caused by different units, CH_4 emission reduction rate or relative mean difference (Eq. 1) was calculated. The relative mean difference (MD) was calculated as follows:

$$\text{Relative MD}=(\text{Treatment mean}-\text{Control mean})/(\text{Control mean})\% \quad (1)$$

The meta-analytical metric included the data of study information, and sample sizes, means of CH_4 emission, standard deviations of treatment group and control group. Due to differences in units of measurements, the standardized mean difference, which is a dimensionless effect measure was calculated (Eq. 2) using the meta package in R statistical software. The default version of standardized mean difference in meta package is Hedges's (g) mean difference which is based on the pooled sample variance and a correction factor for bias (Schwarzer et al., 2015).

$$SMD = (1 - \frac{3}{4n-9}) \frac{MD}{SD_{pool}} \quad (2)$$

where MD is the mean difference, SDpool is the pooled standard deviation, and n is the total sample size of treatment and control on which SDpool is based.

Model fitting

Each group of manure additives described above may contain several different chemicals with similar function (Appendix 2). Therefore, a random-effect model that allows the variance of true effect sizes within each subgroup was used. The random-effect model was fitted to estimate the variance of the distribution of true effect sizes—between-study variance (τ2) and heterogeneity (I2) using the following equation:

$$Y_i = \mu + \zeta_i + \varepsilon_i \quad (3)$$

where Y_i is observed effect, μ is true effect size, ζ_i is true variation in effect sizes, and εi is sampling error. The between-study random effect term ζ_i has the expression of between-study variance Var $(\zeta_i) = \tau^2$ and the sampling error term εi has the expression of sample variance Var $(\varepsilon_i) = s_i^2$. The heterogeneity (I^2) is determined as τ^2 divided by the sum of sample variance and between-study variance ($s_i^2 + \tau^2$) and the I^2 greater than 0.5 indicates significant heterogeneity in general (Dijkstra et al., 2018).

The between-study variability can be modeled either using separate estimates of τ^2 for different subgroups, or a pooled estimate of τ^2 for all subgroups. If the true value of τ^2 varies from one subgroup to another, which is the most likely situation in this analysis, a random-effect model with separate estimates of τ^2 of subgroups should be selected (Borenstein et al., 2011). However, since there was only a few effective studies within some of the subgroups, the separate estimates of τ^2 may be imprecise and the pooled τ^2 is preferable under this situation (Borenstein et al., 2011).

Effect sizes of manure additives

Meta-analyses aim to synthesize evidence from many possible sources, by comparing and combining findings from several studies using statistical methods (Madden and Paul, 2011). The meta-analysis in this review summarizes the effects of manure additives and their potential to reduce CH_4 production in relative terms. Using manure additives to mitigate CH_4 emissions during manure storage has not been as widely applied compared to feed additives, therefore, the number of publications that report on manure additives to control CH_4 emission is much smaller. To increase the sample size for meta-analysis, the manure additives had to be classified into several categories based on their function as mentioned above.

The relative MD of CH_4 emission (%) for each manure additive treatment and the means and standard deviation of CH_4 reduction rates for each type of manure additives were analyzed. The significance test based on grouped manure additives and corresponding *P*-values are listed in Table 5. A summarized box-plot of CH_4 reduction rate for different manure additives is given in Figure 8.

The number of treatments varied considerably for different types of manure additives. Some of the groups such as humate, physical agent, straw and other chemicals contained less than 5 studies each, while acidification and biochar contained over 20 treatments each. Acidification of livestock slurry is considered when the manure additive contains acidic materials that are added to the manure during the storage to lower the pH and inhibit gaseous emissions, including CH_4. It contained different types of acidic component such as aluminum sulfate, sulfuric acid, food industrial waste, phosphogypsum, wood vinegar, etc. The effect of acidification on mitigating NH_3 emission has been widely investigated, and several of the recent observations indicated that CH_4 emissions were also reduced by manure acidification (e.g., Misselbrook et al., 2016; Petersen et al., 2012; Wang et al., 2014). Acidification was the most frequently studied manure additive in our database. Biochar is produced from the thermal decomposition of biomass and it has been applied as manure additive to livestock manure for CH_4 mitigation (Godlewska et al., 2017). Biochar is a cost-effective material with many benefits on manure composting such as enhancing the composting process, improving transformation of nutrient, and reducing the GHG and NH_3 emissions (Mao et al., 2018). In recent years, several types of biochar (cornstalk, bamboo, woody, layer manure, charcoal, holm oak, poultry litter, rice hull, coir and greenwaste biochars) and their effect on gas emissions have been investigated (Agyarko-Mintah et al., 2017; Chen et al., 2017; Chowdhury et al., 2014b; He et al., 2019; Jia et al., 2016; Sanchez-Garcia et al., 2015). A total of 24 valid studies of biochar manure additives were involved in the analysis of CH_4 reduction rate.

Not all manure additives had positive effects on mitigating CH_4 emissions (Figure 8). The relative MD for C/N content, disinfection, masking agent, and oxidizing agent were all greater than zero which indicated the CH_4 emissions of those manure additives treatment groups had increased compared to their control groups even though the increases were not statistically significant ($P > 0.05$) (Table 5). Moreover, the standard deviations of their means were relatively high indicating large variations in mitigation potential of the manure additives. These groups of manure additives were excluded in further analysis.

All other manure additives showed mitigating effects with negative means of CH_4 reduction rate (relative MD). Particularly, the reduction rates of biological mixer, physical agent, straw, and other chemicals for all included studies were less than zero (Max ≤ 0). However, the database contained small sample sizes compared with other categories (N = 3, 3, 4, 2, respectively) (Table 5). The manure additive categories of acidification, biochar, microbial digestion, physical agent, straw, and other chemicals significantly lowered CH_4 reduction rates ($P < 0.05$) and only these manure additives were selected and investigated in the meta-analysis in the next step. There were 6 references containing a total of 18 studies for the 3 manure additives (acidification, biochar and straw) to evaluate the CH_4 emission effect. The studies involved various animal species including swine, poultry, and cattle. The CH_4 emissions reported were either in cumulative or average values during the experimental period with different units. Since the data for each species and manure additives was limited, the species were not evaluated as an effect factor in meta-analysis.

Table 5. Summary statistics of CH_4 reduction rate for different types of manure additives.

Type of Manure Additives	N[a]	Mean[b]	SD	Min	Max	P^c
Acidification	37	-58.9%	30.8%	-98.1%	12.5%	<0.001
Adsorbent	8	-8.8%	15.8%	-34.4%	14.3%	0.160
Biochar	24	-41.3%	51.6%	-85.0%	169.8%	<0.001
Biological mixer	3	-21.5%	37.3%	-64.6%	0.0%	0.423
C/N content	7	32.6%	149.6%	-50.2%	370.0%	0.585
Disinfection	6	124.0%	135.6%	-5.3%	328.6%	0.075
Masking agent	12	221.2%	405.7%	-12.9%	1360.0%	0.086
Humate	3	-8.4%	26.1%	-34.0%	18.2%	0.635
Microbial digestive	12	-33.3%	36.5%	-100.0%	10.5%	0.009
Oxidizing agent	14	60.8%	150.0%	-33.3%	542.9%	0.153
Physical agent	3	-35.6%	9.8%	-46.3%	-27.0%	0.024
Straw	4	-60.1%	26.7%	-100.0%	-45.0%	0.020
Other chemicals	2	-50.0%	5.4%	-53.8%	-46.2%	0.049

[a]N is number of treatments used for the analyses

[b]Mean is the mean reduction rate of CH_4

[c] *P*-value with $\alpha = 0.05$

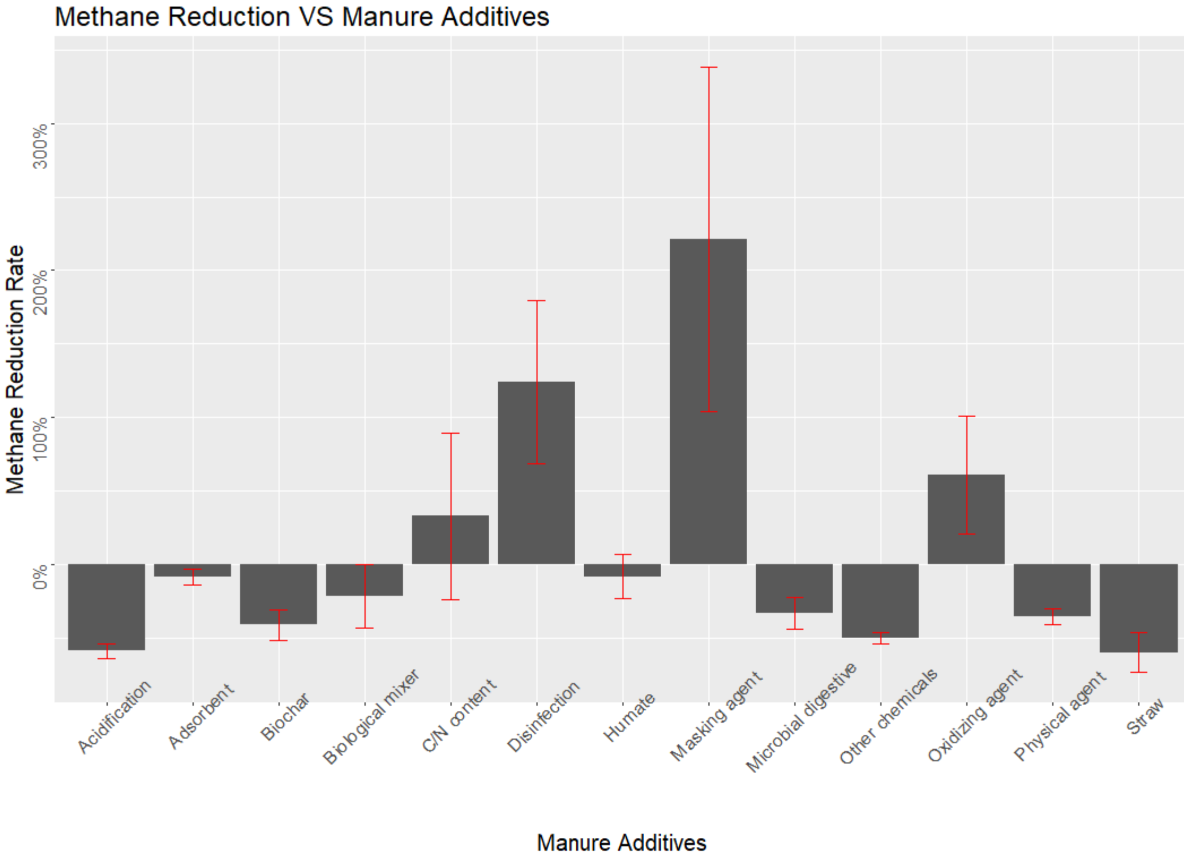


Figure 8. Box-plot of CH_4 reduction rate for different types of manure additives.

Effects of manure additives from random-effects models

The assumption of random-effects model for meta-analyses is that the true effects among all the population of studies are normally distributed and the null hypothesis is that the mean of all relevant true effects is zero (Borenstein et al., 2011). The CH_4 emissions from the 18 studies were significantly reduced by 66.3% on average (Table 6) which were consistent with the SMD from random-effects meta-analysis (P = 0.028). This overall effect indicated that the CH_4 emissions from manure storage could be mitigated by biochar, acidification, and straw. Moreover, the effect of each subgroup manure additives on mitigating CH_4 emissions was also significant with the average reduction rates of 82.4%, 78.1%, and 47.7%, respectively (P < 0.05). The most effective manure additive was biochar followed by acidification and straw according to relative MD analysis. Biochar as a manure additive also showed the greatest effect (SMD = -2.72), followed by straw (SMD = -1.86) and acidification (SMD = -1.31) based on the SMD of random-effects model. The SMD estimates with 95% CI according to each subgroup effects is presented using forest plot (Figure 9). The total observations of acidification subgroup were much larger compared to the other additives. The 95% CI of manure additives effects in acidification subgroup were all positive, however, the SMDs of studies from Regueiro et al. (2016) and Samer et al. (2014) varied considerably between -0.6 and -37.0. The total weights of acidification subgroup accounted for over 60% among all manure additives in the overall effect estimates, while the biochar and straw subgroups accounted for 28% and 11.9%, respectively (Table 5). This unbalanced distribution of studies' weight in the overall effect size might generate bias among different manure additives.

Table 6. Effect size and heterogeneity estimates based on overall and subgroup random-effect models.

Variable and model	Relative MD		SMD and Heterogeneity					
	MD ± SE[a]	P[b]	SMD	Weight (%)	P[b]	τ^2	I^2 (%)	P[b]
REM[c]								
Overall effect	-0.663 ± 0.006	<0.001	-1.732	100	0.028	19.4	21.8	0.195
REM[c]--Subgroup								
Acidification	-0.781 ± 0.025	<0.001	-1.311	60.1		21.8	0.0	Between group: 0.564
Biochar	-0.824 ± 0.020	<0.001	-2.721	28.0		21.8	23.8	
Straw	-0.477 ± 0.023	0.030	-1.862	11.9		21.8	0.0	

[a] SE = Standard error corresponding to number of studies for each group

[b] P-value with $\alpha = 0.05$

[c] REM = random-effect model

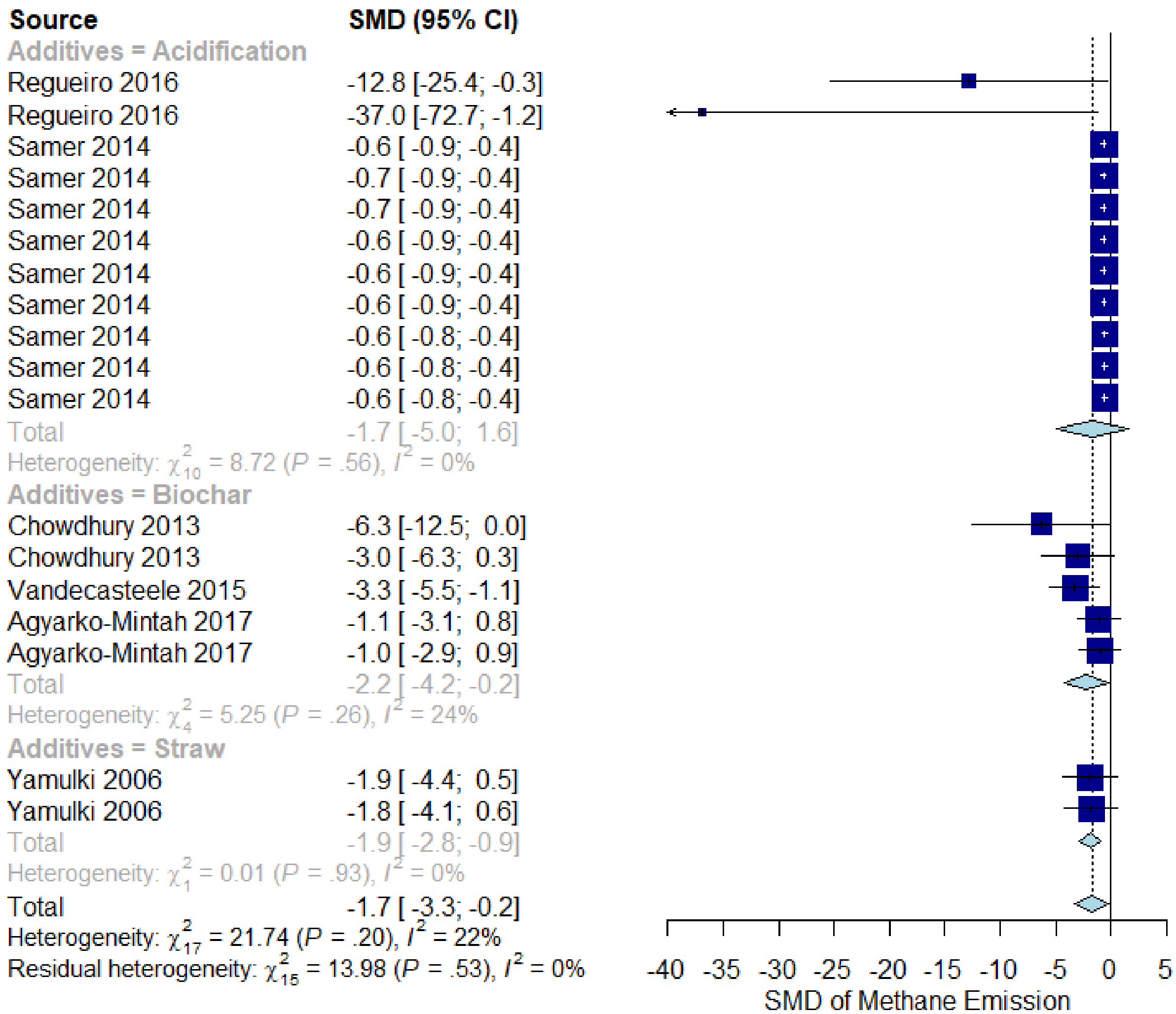


Figure 9. Forest plot showing standardized mean difference, and 95% confidence interval for three selected manure additives.

Heterogeneity test

The heterogeneity of the overall random-effects model was quantified using τ^2 and I^2. The effects of manure additives were associated with non-significant heterogeneity across all the three manure additives with only 21.8% of the total variability of the effect of manure additives in mitigating CH_4 was due to heterogeneity. As mentioned in model fitting section, a pooled τ^2 is a more precise method to conduct the random-effects model because the sample size of useful studies for manure additives was small. Therefore, the three subgroups shared the same τ^2 (21.8; Table 6). The between-study variability was not significant among the three manure additives (P = 0.564) and for the subgroups test, the heterogeneity of acidification and straw subgroups were both zero, which suggested little heterogeneity. The effects of biochar were associated with 23.8% of heterogeneity, however, this variability due to the heterogeneity was not significant (P = 0.26; Figure 9). Therefore, the heterogeneity test indicated the random-effects model was appropriate to use to quantify effect of manure additives CH_4 reduction. Mitigating CH_4 emissions by applying certain manure additives was an effective method but depends on the type of additives.

Analysis of manure type, additive type, and characteristic of treatment manure

The composting manure from livestock were categorized into manure type (cattle, poultry, swine, mixture of different manure types) and manure additives described in Table 7 and their characteristics of composting manure including pH, C/N ratio, and moisture content were generally analyzed. The initial means of pH (7.2-7.9), C/N ratio (11-18), and moisture content (58.0-75.5) for different manure types not varied in large ranges, however, the differences of means between additive types were visible. Most of the raw manure were weakly alkaline, but the averaged pH for manure composting with acidic additives (6.2 ± 1.33) was lower than other types due to the reaction of acidification. The mean of C/N ratio for biochar was relatively greater (23.6 ± 9.17) because most of the biochar contained and was made by high carbon materials such as bamboo biochar, charcoal, and cornstalk (Chowdhury et al., 2013; Chen et al., 2017; Liu et al., 2017). The highest mean C/N ratio was observed in cattle manure, and the lowest was in poultry manure, with the moderate one in swine manure which associated with the N content in the raw material. These findings showed consistency with Cao et al. (2019). Moisture content usually associated with the porosity of the material and Richard et al. (2002) indicated that moisture content for an optimum performance of composting process may vary widely between 50 to over 70% based on different raw material and composted times. All of the averaged moisture contents for different manure types and additive types were within the range of 50-75%, except for the adsorbent and biological.

A general linear regression analysis for CH_4 mitigating rate [(CH_4 emission in treatment group – control group)/control group] response to pH, C/N ratio, moisture content, manure type (cattle, poultry, and swine), and additive type (acidification, biochar, biological, physical, and C/N content) were conducted (Table 8) with a partial data from Appendix 2 which included 11 articles with 37 studies that contained completing information of all variables and CH_4 emissions (Hao et al., 2005; Yamulki, 2006; Chowdhury et al., 2013; Luo et al., 2013; Samer et al., 2014; Vandecasteele et al., 2016; Agyarko-Mintah et al., 2017; Chen et al., 2017; Liu et al., 2017; Owusu-Twum et al., 2017; Zhang et al., 2017). Moisture content significantly related to the CH_4 mitigation rate, and higher moisture content enhanced the overall reduction of CH_4 emissions (P = 0.01), however, the CH_4 reduction rate was not significant affected by pH (P = 0.731) and C/N (P = 0.218) in a linear manner (need to find a reasonable explanation). Manure types (P > 0.531) did not make significant impacts on CH_4 mitigation from composting process, however, some additives types showed significant differences from others, such as acidification vs. biological (P = 0.001), acidification vs. physical (P = 0.017), and biochar vs. biological (P = 0.013).

Table 7. Summary of livestock manure type, additives type, number of observations (N) and the characters of manure pH, C/N ratio, moisture content reported in mean and standard deviation.

Item		pH	N	C/N	N	Moisture content (%)	N
Manure Type	Cattle	7.2 ± 0.46	35	18 ± 4.08	8	75.5 ± 19.34	11
	Poultry	7.9 ± 0.66	8	14.6 ± 4.54	6	62.8 ± 13.45	8
	Swine	7.7 ± 0.30	10	17.8 ± 0.41	4	58.0 ± 14.12	9
	Mixture	NA	NA	11.0	1	69.0	1
Additives Type	Acidification	6.2 ± 1.33	33	20.7 ± 3.71	22	73.4 ± 19.74	33
	Adsorbent	7.2 ± 1.24	2	NA	NA	36.2	1
	Biochar	7.9 ±0.68	16	23.6 ±9.17	15	58.8 ±12.21	18
	Biological	6.9 ± 0.03	2	16.4 ± 0.00	2	43.4 ± 21.42	3
	C/N content	8.0 ± 0.13	4	14.2 ± 3.23	8	64.1 ± 2.49	10
	Disinfection	7.8 ± 0.30	3	NA	NA	NA	NA
	Masking agent	7.3 ± 0.11	6	NA	NA	NA	NA
	Humate	7.4 ± 0.02	3	NA	NA	NA	NA
	Microbial digestive	7.4 ± 0.54	5	NA	NA	NA	NA
	Oxidizing agent	7.0 ± 0.17	7	NA	NA	NA	NA
	Physical agent	8.6 ± 0.15	3	18.7 ± 1.51	3	63.0 ± 1.66	3
	Straw	8.1 ± 0.14	2	17.3 ± 2.89	3	61.7 ± 3.35	3
	Other chemicals	NA	NA	NA	NA	69.0	1

Table 8. Linear regression analysis of response CH4 mitigation rate vs. pH, C/N content, and moisture content.

Factor[1]	Mean[2]	SE[3]	*P*-value
CH_4 mitigation rate (%)	-32.2	46.77	0.496
pH	1.46	4.201	0.731
C/N	0.945	0.7516	0.218
Moisture content (%)	-0.802	0.2916	0.010

[1]Manure type: $P > 0.531$; Additive type: acidification vs. biochar $P = 0.055$, acidification vs. biological $P = 0.001$, acidification vs. physical $P = 0.017$; biochar vs. biological $P = 0.013$; biological vs. C/N content $P = 0.064$; all others $P > 0.100$.
[2]Mean of n = 37.
[3]SE = standard error.

Conclusions

Studies investigating manure additives for reducing CH_4 emission during storage and composting are scarce. Manure additives that include acidification, biochar, microbial digestion, physical agent, straw, and other chemicals significantly reduced CH_4 emissions from manure. In general, higher moisture contents in raw composting manure could enhance the CH_4 mitigation rates, however, the pH, and C/N content were not linearly related to CH_4 mitigation. Adding biochar, acids, and straw to manure could mitigate CH_4 emissions by 82.4%, 78.1%, and 47.7%, respectively. However, the data for straw is quite small so it should not be taken out of context as it may introduce a source of carbon into lagoons. The meta-analysis conducted with selected additives indicated manure additives were an effective method to reduce CH_4 emission, with biochar being the most effective. However, further studies of manure additives on CH_4 mitigation are required to support a more accurate quantitative analysis and potential impacts to water quality and crop yield after land application. Most of the research for biochar and straw is when used as additive to solid or semi solid manure so they should be interpreted in that context.

NET REDUCTIONS IN GREENHOUSE GASES FROM FEED ADDITIVES IN CALIFORNIA

A review of feed additives that can potentially be used in California revealed that 3NOP and nitrate may have the potential to be used as there is enough evidence of their effectiveness. Several other additives including Mootral, macroalgae and Agolin also have the potential but further studies are required to determine levels of effectiveness, safety and adequate sourcing. There was only one publication dealing with Mootral in California. Therefore, this section aims to estimate the net GHG emissions in California dairy system based on supplementation of 3NOP and nitrate to the basal diet. The following narrative will be submitted for publication to *California Agriculture*.

Materials and methods

The study was based on a life cycle assessment (LCA) conducted for the dairy industry in California (Naranjo et al., 2020). The feed ingredients used by Naranjo et al. (2020) were adjusted and recalculated using NRC (2001). The impact of producing the feed additives 3NOP and nitrate was integrated in the LCA model. Energy corrected milk (ECM) was used as the functional unit and all emissions were calculated and standardized to 1 kg of ECM.

The milk production supply chain in California from cradle to farm gate was considered the system boundary of the LCA including production of the feed additives. Specifically, these include: crop production, feed additives production, farm management, enteric methane, and manure storage. The system boundary considered emissions associated with on-farm activities, pre-farm production, and transportation of major productions up to the animal farm gate. Emissions for further activities after the products left the farm gate were not accounted in the system because they were considered to be treated in the same way for all scenarios.

Mitigation scenarios

Data sources collected from USDA National Agricultural Statistical Service (USDA-NASS) and Economic Research Service (USDA-ERS), California Department of Food and Agriculture (CDFA), peer-reviewed literature and other published resources, and databases generated from GaBi 6 software were summarized and used based on the priority of data accuracy (Naranjo et al., 2020). The GHG emissions from each process in the LCA were estimated based on the average conditions (Model 2) for dairy cattle in California as described by Naranjo et al. (2020).

The control scenario used representative diets for the California dairy cows collected from the reports by CDFA. Averaged data from 2013 to 2015 represented the diets for year 2014 in the current analysis. Within each reference year, the diets for dairy cows at different growth stages including calf up to 1 year, heifer, pregnant heifer, close-up heifer, high lactating cow, and dry cow were weighted based on a whole production cycle. We assume 4 lactations to be the average life span of a California dairy cow. The crop production for control scenario included the activities related to producing feed, and use of land, water, fertilizers, pesticides and herbicides. Additionally, energy used for machine operation, irrigation, and transportation was included. Data from USDA-NASS Quick Stats (USDA–NASS, 2017), USDA farm and ranch irrigation reports (USDA 2013), California specific agricultural reports (Burt et al., 2003; Johnson and Cody, 2015), USDA-ERS reports (USDA–ERS, 2011), University of California crop cost and return studies (UC Agricultural Issues Center, 2016), and values published in literatures (Liedke and Deimling, 2015) were used to estimate the emissions during the crop production. Enteric CH_4 emissions, farm management, energy and water used for producing crop, feeding cattle, cooling livestock facilities, animals, and milk, sanitation, cleaning, and dealing with onsite waste were according to

Naranjo et al. (2020). Similarly, manure methane and nitrous oxide (N_2O) emissions were based on methodology described by Naranjo et al. (2020).

Two scenarios were developed to estimate net mitigation effect of supplementing 3NOP to typical dairy diet in California. In scenario 1, all dairy cows were simulated to consume a diet that contains 3NOP only during lactation. In scenario 2, 3NOP was supplemented to the diet at all growing stages within a life cycle. The basal diets were same as in the control scenario and 3NOP was supplemented at a rate of 127 mg/kg DM in both scenarios.

Nitrate as a non-protein nitrogen source for cattle is usually used to replace other non-protein N sources such as urea (Velazco et al., 2014; Rebelo et al., 2019). Urea is not typically used as a nitrogen source in California representative diets, so nitrate was simulated to partially replace dietary true protein in diets to keep similar N supply for all nitrate scenarios. In nitrate scenario 1, all dairy cows were simulated to consume a diet that contained nitrate only during lactation. Nitrate was supplemented to dairy cows at all stages in nitrate scenarios 2 and 3. In nitrate scenario 2, high protein meal (e.g. corn gluten, soybean meal, and DDGS) was replaced by dietary nitrate on an equivalent N basis with no adjustment for DMI. In nitrate scenario 3, DMI was adjusted using low protein meal (e.g. corn grain, and wheat silage) to the control levels after replacing high protein meal with nitrate additives. Nitrate was supplemented to dairy cattle at a rate of 17.7 g/kg of DM for all the 3 nitrate treatment scenarios.

Emission associated with production and use of additives

3-Nitrooxypropanol

The carbon footprint of emissions associated with 3NOP production were assumed to be 52 kg CO_2e/kg 3NOP produced (DSM Nutritional Products, Ltd., pers. comm.). Moreover, with the improvement of process optimization, the carbon footprint of 3NOP could drop to 35 kg CO_2e/kg 3NOP (DSM Nutritional Products, Ltd., pers. comm.). The total GHG emissions from 3NOP production were estimated using both of the factors and the results were reported as mean with standard error to evaluate the effect of 3NOP emissions factors on total emissions. The transportation of 3NOP was calculated based on shipping from the producer (DSM Nutritional Products, Ltd., registered in Ontario, CA) to dairy farms in California by truck. The average distance used to estimate the emissions related to 3NOP transportation was weighted according to the milk production amount in California counties in 2014 (CDFA, 2014).

The magnitude of enteric CH_4 emission reduction as a result of supplementing 3NOP was calculated based on an updated version of a meta-analysis conducted by Dijkstra et al. (2018) on the anti-methanogenic effects of 3NOP. Four more recent references related to 3NOP effect on CH_4 emissions were added to the previous analysis to extend the accuracy and robustness of the meta-analytical model. The updated database included treatment means from Martinez-Fernandez et al. (2018) (beef; 1 treatment), Vyas et al. (2018) (beef; 2 treatments), Kim et al. (2019) (beef; 4 treatments), and van Wesemael et al. (2019) (dairy; 2 treatments). The final mixed-effect models for CH_4 production in the updated meta-analysis indicated effectiveness of 3NOP at mitigating CH_4 production was positively associated with 3NOP dose, and negatively associated with NDF content. Similar to the previous study, supplementation of 3NOP had stronger anti-methanogenic effects in dairy cows compared to beef cattle, at a slightly greater magnitude of mitigation. The following equations were used to calculate the mitigation effect of 3NOP that includes dose, NDF content and either dairy (Equation 1) or beef (Equation 2):

$$\text{Enteric methane reduction rate (\%)} = -41.5 - (0.260 \times \text{3NOP dose}) + (0.129 \times \text{NDF content})$$

Equation 1

$$\text{Enteric methane reduction rate (\%)} = -22.8 - (0.260 \times \text{3NOP dose}) + (0.129 \times \text{NDF content})$$

Equation 2

The equations were centered on the mean values of 127 mg 3NOP /kg DM and 326 g NDF /kg DM. Therefore, the methane reduction rates were adjusted for each cattle type when the NDF content in the 3NOP supplemented scenarios varies from the default centered value. The NDF contents for different growing stages of dairy cows in California used in this study were calculated using NRC (2001) based on ingredients supplied (Table 9). In 3NOP scenario 1, enteric CH_4 emitted from lactating cows was reduced by 38.8%, which includes adjustment for NDF content (Table 9). In scenario 2, if the cows were not lactating, the emission reduction rate was assumed to be similar to beef cattle so Equation 2 was applied. The enteric CH_4 reduction rates for heifer, pregnant heifer, close up heifer, high lactating cow, and dry cow were 11.1%, 1.1%, 10.3%, 38.8%, and 4.0%, respectively (Table 9).

The GHG emissions from the farm management and manure management processes in the LCA for 3NOP scenarios were same as for the control scenario because we assumed no residues and by-products from the 3NOP production process. Nkemka et al. (2019) confirmed that there was no residual effect on anaerobic digestion of the manure from beef cattle fed diets supplemented with 3NOP.

Table 9. Enteric methane reduction rates and total emissions per life cycle at different dairy growing stages for control and treatment scenarios.

Cattle Stage	Control		3NOP 1[a]		3NOP 2[a]		Nitrate 1		Nitrate 2		Nitrate 3	
	Reduction (%)	CH_4 (kg/lifetime)	Reduction (%)	CH_4 (kg/lifetime)	Reduction (%)	CH_4 emission (kg/lifetime)	Reduction (%)	CH_4 (kg/lifetime)	Reduction (%)	CH_4 (kg/lifetime)	Reduction (%)	CH_4 (kg/lifetime)
Calf	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Heifer	0	10.6	0	10.6	-11.1	9.4	0	10.6	-15.4	9.1	-15.4	9.6
Pregnant	0	73.8	0	73.8	-1.1	72.9	0	73.8	-15.4	62.3	-15.4	66.0
Close up	0	9.2	0	9.2	-10.3	8.2	0	9.2	-15.4	7.6	-15.4	7.8
lactating	0	575.8	-38.8	352.4	-38.8	352.4	-15.4	481.7	-15.4	481.7	-15.4	488.5
Dry cow	0	60.9	0	60.9	-4.0	58.5	0	60.9	-15.4	50.0	-15.4	52.0

aNDF content (g/kg DM) in diets for 3NOP scenarios: 250 (Calf up to 1 year), 419 (Heifer), 496 (Pregnant heifer), 425 (Close up heifer), 349 (High lactating cow), and 474 (Dry cow).

Nitrate

Nitrate was assumed to be supplemented to dairy diets as Calcium nitrate ($Ca(NO_3)_2$). Brentrup et al. (2016) reported carbon footprint associated with $Ca(NO_3)_2$ production were estimated to be 1.76 kg CO_2e/kg $Ca(NO_3)_2$ in USA and 0.67 kg CO_2e/kg $Ca(NO_3)_2$ produced in Europe. Total emissions associated with $Ca(NO_3)_2$ production were calculated using both carbon footprint values for USA and Europe, and the emissions from nitrate production process are reported as the mean with standard deviation. Emissions related to transportation of $Ca(NO_3)_2$ was calculated based on the shipping distance between supplier and dairy farms in California. Several chemical companies supply $Ca(NO_3)_2$ within California and the plant with the minimum travel distance (by truck) to each county was assumed as its $Ca(NO_3)_2$ supplier. The overall average distance was weighted based on the milk production in California counties in 2014 (CDFA, 2014) and used for emission calculations related to chemical transportations. Feed production for different nitrate treatment scenarios were recalculated based on the replacement of high protein meals by dietary nitrate to provide equivalent N as compared to the diets for control scenario at each growing stage using NRC (2001) software.

The anti-methanogenic effects of nitrate were calculated based on equations developed by Feng et al. (2020 unpublished). Meta-analytical results indicated nitrate effect on enteric CH_4 production to be significantly affected by nitrate dose. However, there was no difference in effectiveness in dairy and beef cattle. The reduction rate for enteric CH_4 emissions is estimated by the meta-analytical model as given in Equation 3.

$$\text{Enteric methane reduction rate (\%)} = -14.6 - (0.808 \times \text{nitrate dose}) \qquad \text{Equation 3}$$

The equation is centered on mean nitrate dose of the database, which was 17.7 g/kg of DM. We kept the average as the dose of nitrate supplementation in the scenarios evaluated in this study.

We assumed there were no residues and by-products from nitrate production and the total GHG emissions from farm management process for nitrate treatment scenarios including on-farm energy and water usage were not affected by nitrate additives. Methane emissions from manure storage were calculated as a function of VS (Nielsen et al., 2013) which was associated with NDF content, CP content and DMI (Appuhamy et al., 2016). As the dietary ingredients and DMI for nitrate scenarios varied with the adjustment of nitrate additives, the total GHG emissions from manure management were recalculated based on the different nitrate feeding scenarios.

Results and discussion

3-Nitrooxypropanol

The GHG emissions from crop production, farm management, enteric CH_4 and manure storage for control scenario were 0.174, 0.0608, 0.432, and 0.457 kg CO_2e per kg of ECM produced in California, respectively (Figure 10). Total GHG emissions from crop production, farm management, and manure storage were not affected by feeding 3NOP to dairy cows. The mean GHG emissions related to production of 3NOP in scenario 1 was 3.23 g CO_2e/kg ECM which was lower than 3.92 g CO_2e/kg ECM in scenario 2 because 3NOP was only fed to lactating cows in scenario 1. Enteric CH_4 emissions were 0.298 and 0.295 kg CO_2e/kg ECM for 3NOP scenarios 1 and 2, respectively, which were reduced by 31.0% and 31.7% compared to the control scenario, respectively, due to the inhibition effect of 3NOP on CH_4 production. Accounting for 3NOP production, the net enteric methane emission reduction was 30.3% in scenario 1 and 30.8% in scenario 2.

The total GHG emissions for control and 3NOP treatment scenarios 1 and 2 were 1.12, 0.993 and 0.991 kg CO_2e/kg ECM, respectively (Figure 10). Feeding 3NOP to dairy cows resulted in a net reduction of total GHG emission of 11.3% in 3NOP scenario 1 and 11.5% in 3NOP scenario 2 compared to the control scenario. Using 3NOP for dairy cows at all growing stages only further reduced 0.2 percentage points more compared to limiting 3NOP supplementation during lactation.

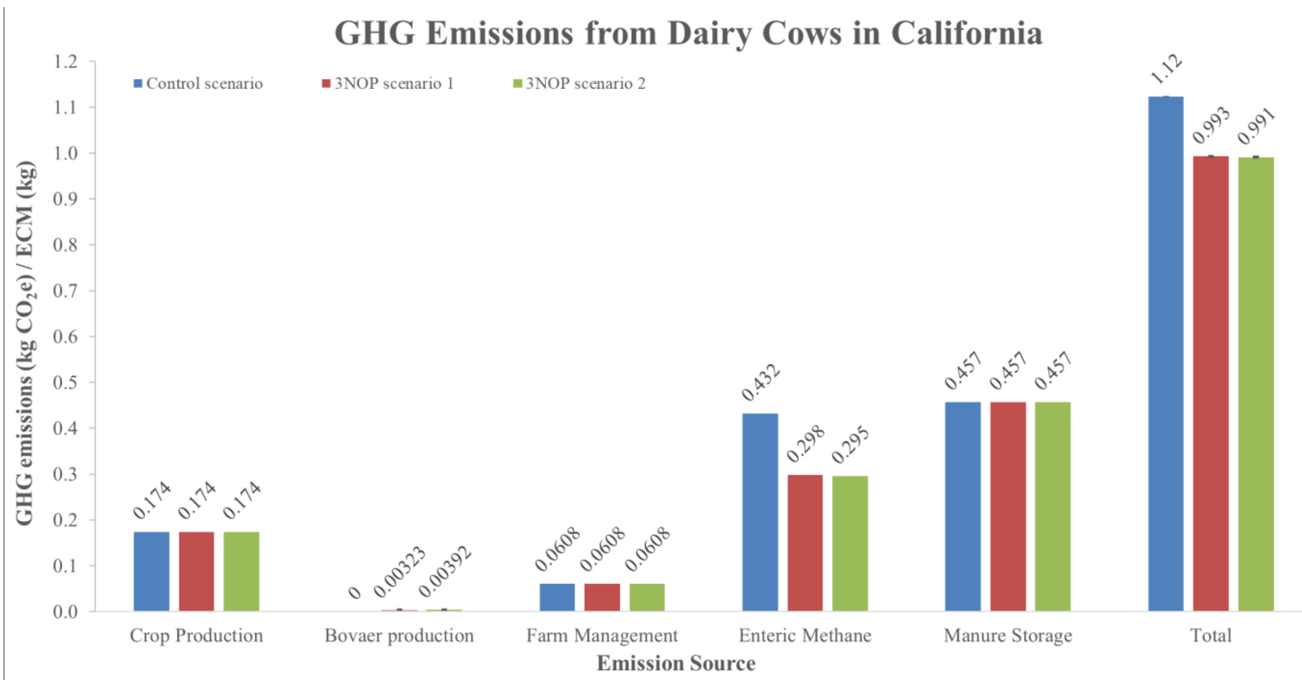


Figure 10. Comparison of global warming potential (GWP) by emission source for control and 3NOP scenarios 1 and 2 in California dairy cows.

The GHG emissions associated with 3NOP production for scenarios 1 and 2 were 3.86 and 4.69 g CO_2e/kg ECM, respectively, assuming 3NOP carbon footprint of 52 kg CO_2e/kg and 2.60

and 3.16 g CO_2e/kg ECM, respectively, using manufacturer reported values of 35 kg CO_2e/kg 3NOP. This indicates that with the improvement of manufacturing process, the GHG emissions from 3NOP production can be reduced by 32.6%, improving net impact of 3NOP in reducing enteric emissions.

Nitrate

The total GHG emissions and estimates of the various components in dairy cattle supplemented with nitrate is given in Figure 11. In nitrate scenario 1, the mean GHG emissions associated with nitrate production was 0.0182 kg CO_2e/kg ECM and 0.0219 kg CO_2e/kg ECM in nitrate scenarios 2 and 3 due to differences in the phases of dairy production that nitrate was included. The error bars for nitrate production in Figure 11 showed the deviations of GHG emissions estimated with different carbon footprint of $Ca(NO_3)_2$ production in USA and Europe. According to Brentrup et al. (2016) the difference was mainly due to a catalyst technology developed in Europe. The GHG emissions calculated with carbon footprint value for $Ca(NO_3)_2$ in USA were 0.0255 kg CO2e/kg ECM for nitrate scenario 1, and 0.0307 kg CO2e/kg ECM for nitrate scenarios 2 and 3. Using the European carbon footprint (0.67 kg CO2e/kg $Ca(NO_3)_2$ produced), the GHG emissions from nitrate production was 0.0109 kg CO2e/kg ECM for nitrate scenario 1, and 0.0131 kg CO2e/kg ECM for nitrate scenarios 2 and 3. The GHG emissions from nitrate production decreased 57.3% based on European values compared to those in USA.

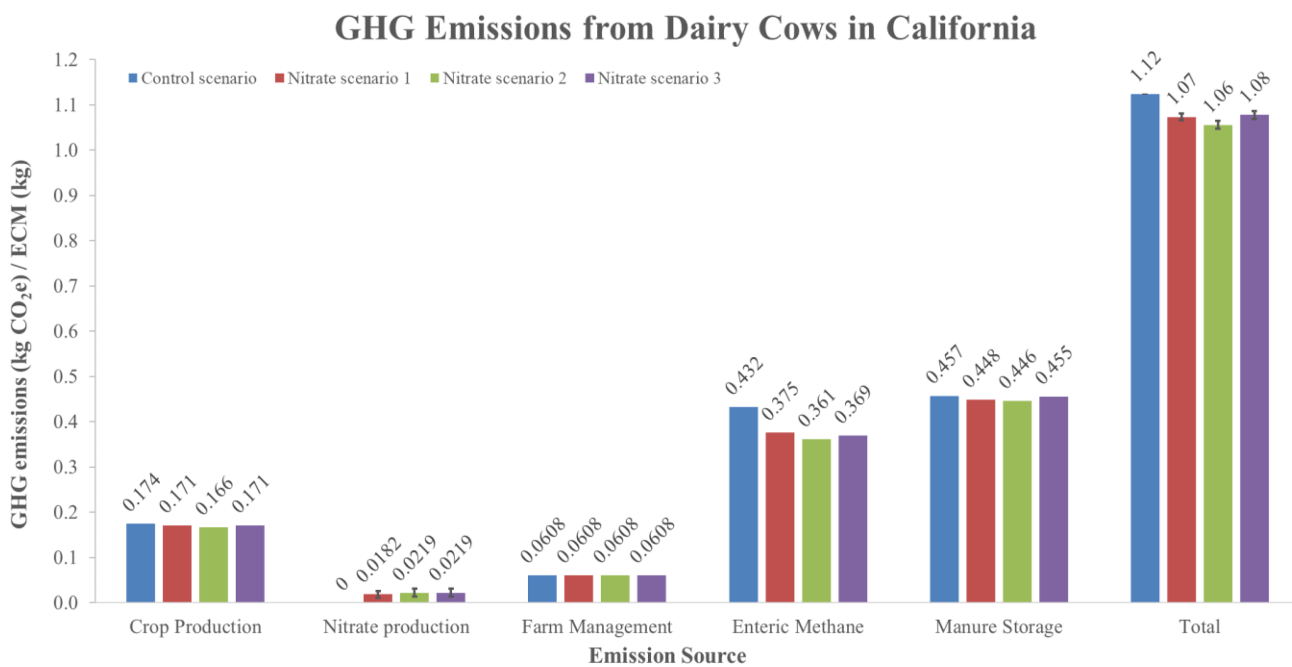


Figure 11. Comparison of global warming potential (GWP) by emission source for control and nitrate scenarios of dairy cows in California.

The GHG emissions related to crop production was 0.174 kg CO_2e/kg ECM for the control scenario, and reduced to 0.171, 0.166, and 0.171 CO_2e/kg ECM for nitrate scenarios 1, 2, and 3, respectively, which was mainly caused by the decline in the amount of protein that was replaced by nitrate. The DMI for scenario 3 was adjusted back to the control level, and therefore the GHG emissions from crop production in nitrate scenario 3 was 0.005 CO_2e/kg ECM greater than in scenario 2. The GHG emissions from manure storage were 0.457, 0.448, 0.446, 0.455 kg CO2e/kg ECM in control and three nitrate scenarios, respectively. The differences of GHG emissions from manure management among nitrate scenarios were associated with the variations in dietary NDF content, CP content, and DMI of adjusted diets. Enteric CH_4 emissions from nitrate scenarios 1 to 3 were 0.375, 0.361, and 0.369 kg CO_2e/kg ECM respectively, which were reduced by 13.2%, 16.4%, and 14.6% respectively, compared to CH_4 emissions from control scenario (0.432 CO_2e/kg

ECM) based on values calculated for CH_4-mitigating effect of dietary nitrate (Table 9). The net reduction enteric methane emission (including nitrate production) is calculated to be 8.98, 11.35 and 9.51% for nitrate scenarios 1 to 3, respectively. The GHG emissions from farm management were the same for control and all nitrate scenarios which was 0.0608 kg CO_2e/kg ECM (Figure 11).

The total GHG emissions for control scenario was 1.12 kg CO_2e/kg ECM, while with supplementing dietary nitrate to dairy cows in California, the total GHG emissions were 1.07, 1.06, and 1.08 kg CO_2e/kg ECM respectively in nitrate scenarios 1, 2, and 3. Therefore, the total GHG emissions for three nitrate scenarios were reduced by 4.5%, 5.4%, and 3.6% from the control scenario. The net reductions of total GHG emissions for nitrate scenarios 1, 2, and 3 were 0.05, 0.06, 0.04 kg CO_2e/kg ECM, respectively (Figure 11). Nitrate scenario 2 showed the greatest net reduction of total GHG emissions which reduced 0.9% and 1.8% more of total GHG emissions compared to scenarios 1 and 3, respectively.

Comparison of 3-nitrooxypropanol and nitrate additives

Total GHG emissions from control scenario were lower than values published in several previous studies. For example, Gerber et al. (2011) reported the GHG emissions in North America to be 1.20 kg CO_2e/kg ECM and Thoma et al. (2013) reported 1.23 kg CO_2e/kg ECM. In Canada, Alvarez-Hess et al. (2019) reported 1.21 kg CO_2e/kg ECM, but in two Australian dairy farms, the authors reported 1.09 and 0.97 kg CO_2e/kg ECM, respectively, which were slightly lower than the value estimated in the present study. Emissions from manure storage accounted for 40.6% to 46.1% of the total GHG emissions, which contributed the largest amount to total GHG emissions in all scenarios. Enteric CH_4 emissions from control scenario accounted for 38.4% of the total GHG emissions but the proportions of enteric CH_4 emissions dropped and varied between 29.8%

(3NOP, scenario 2) to 35.0% (nitrate scenario 1). Crop production emitted 15.5% to 17.6% of total GHG emissions and the significant decrease in enteric CH_4 emissions resulted in a proportional increase of GHG emissions of crop production in 3NOP scenarios. Only 0.3% to 2.1% of emissions were attributed to feed additives production in supplemental scenarios. The GHG emissions associated with farm management were same for all scenarios.

Although both 3NOP and nitrate additives decreased the total GHG emissions, the mitigating effect of 3NOP was greater than nitrate reaching a highest reduction rate of 11.8% (3NOP scenario 2). The average net reduction rate of GHG emissions for 3NOP was 11.7% and supplementing 3NOP to dairy cows only during lactations or to the entire growing herds had a minor difference in the total GHG emissions. The mean net reduction rate of GHG emissions in dairy cows feeding nitrate was 4.9%. The greatest net GHG emissions achieved with nitrate was 6.1% with supplementation of nitrate to dairy cows in all growing stages. These results partially agreed with Alvarez-Hess et al. (2019) who reported that the GHG emissions went down from 1.13 kg CO_2e/kg ECM to 1.10 kg CO_2e/kg ECM (a reduction of 2.65%) when nitrate was fed to lactating cows only at a rate of 21 g/kg DM. The GHG emissions from groups supplemented with 3NOP at 86 mg/kg DM were between 0.83 and 1.03 kg CO_2e/kg ECM in dairy farms in Australia and Canada (Alvarez-Hess et al., 2019).

The carbon footprint of nitrate is greater than that of 3NOP and it is fed at a rate of an average 17.7 g/kg DM compared to an average of 127 mg/kg DM for 3NOP. Therefore, much higher quantities for nitrate are required for methane mitigation resulting in about 5.6 times GHG emission from production of the additive. Moreover, nitrate toxicity caused by the high methemoglobin levels in ruminants fed in greater quantities is a concern and currently not recommended as methane mitigating feed additives to cattle (Bruning-Fann and Kaneene, 1993; Lee and Beauchemin, 2014).

The impact of manure additives can be added to the effect of feed additives. When biochar, acids, and straw are used alongside 3NOP the potential combined effect would be 20 to 34% from the whole dairy production system in CA.

Conclusions

This LCA was conducted based on dairy cows in California and evaluated the mitigation effect of two promising feed additives—3NOP and nitrate, on total GHG emissions. The average net reduction rate of supplementing 3NOP and nitrate were 11.7% and 4.9%, respectively. 3NOP had a greater effect than nitrate on reducing total GHG emissions with a highest performance of 11.8%. Feeding 3NOP to only lactating cows or to the entire growth stages did not make significant difference in total GHG emissions. Considering California milk production of 18 billion kg in 2017, using nitrate on California dairy cows would reduce GHG emissions 1.09 billion kg CO_2e and 3NOP 2.33 billion kg CO_2e annually.

SUMMARY

This study evaluated strategies to reduce methane emission from enteric and lagoon sources with emphasis on California conditions. A considerable amount of literature is available on feed additives but studies on manure additives are much more scarce. Through a literature review, a large amount of feed additives were considered, but only about 17% of those evaluated through effect size analysis had a statistically significant mitigating impact on methane emissions. The majority of those were found to either increase cost, reduce productivity or increase an alternative pollutant at the expense of methane mitigation. Therefore, only 3NOP and nitrate were identified as those with the highest potential. An updated meta-analysis for effectiveness of 3NOP showed 41% reduction in dairy cattle and 22.4% in beef cattle. A new meta-analysis for nitrate showed 14.4% reduction in mitigating methane with no differences between dairy and beef cattle. In both cases dosage of feed additives was related to further reduction in emissions.

Manure additives that include acidification, biochar, microbial digestion, physical agent, straw, and other chemicals significantly reduced CH_4 emissions from manure. In general, higher moisture contents in raw composting manure could enhance the CH_4 mitigation rates, however, the pH, and C/N content were not linearly related to CH_4 mitigation. Adding biochar, acids, and straw to manure could mitigate CH_4 emissions by 82.4%, 78.1%, and 47.7%, respectively. The meta-analysis conducted with selected additives indicated manure additives were an effective method to reduce CH_4 emission, with biochar being the most effective. However, further studies of manure additives on CH_4 mitigation are required to support a more accurate quantitative analysis. A life cycle assessment was conducted based on dairy cows in California and evaluated the mitigation effect of 3NOP and nitrate on total GHG emissions.

The average net reduction rate of supplementing 3NOP and nitrate were 11.7% and 4.9%, respectively. 3NOP had a greater effect than nitrate on reducing total GHG emissions with a highest performance of 11.8%. Feeding 3NOP to only lactating cows or to the entire growth stages did not make significant difference in total GHG emissions. Given the toxicity concerns of nitrate, only 3NOP is recommended for use pending FDA approval. However, further research is highly recommended for Mootral, macroalage and grape pomace to establish efficacy and solve related issues as there were only one or two studies conducted relevant to California conditions.

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Appendix 1. Number of treatment, mean, standard deviation, minima and maxima of mean difference of methane production for control and treatment groups based on feed additive type

Treatment Type	Col/Trt	counts	meanMD	sdMD	maxMD	minMD
3NOP	Control	8	0.0	0.00	0.0	0.0
3NOP	Treatment	15	-71.8	71.13	-1.2	-212.1
Acacia mearnsii	Control	5	0.0	0.00	0.0	0.0
Acacia mearnsii	Treatment	6	-45.4	45.06	-1.2	-126.0
Acetate inclusion	Control	2	0.0	0.00	0.0	0.0
Acetate inclusion	Treatment	2	-11.3	2.13	-9.8	-12.8
Antibloat	Control	1	0.0	NA	0.0	0.0
Antibloat	Treatment	1	-103.2	NA	-103.2	-103.2
Bacteria	Control	14	0.0	0.00	0.0	0.0
Bacteria	Treatment	14	4.5	7.81	23.0	-3.1
Bromochloromethane	Control	10	0.0	0.00	0.0	0.0
Bromochloromethane	Treatment	20	-32.8	27.07	-0.8	-89.7
Calcium soap inclusion	Control	2	0.0	0.00	0.0	0.0
Calcium soap inclusion	Treatment	4	-8.6	12.04	9.3	-16.5
Camelina inclusion	Control	1	0.0	NA	0.0	0.0
Camelina inclusion	Treatment	1	-120.0	NA	-120.0	-120.0
Canola inclusion	Control	2	0.0	0.00	0.0	0.0
Canola inclusion	Treatment	2	-34.4	23.90	-17.5	-51.3
Carboxylic acid	Control	5	0.0	0.00	0.0	0.0
Carboxylic acid	Treatment	5	1.1	4.70	5.5	-6.0
Cerium chloride	Control	1	0.0	NA	0.0	0.0
Cerium chloride	Treatment	3	-4.2	2.18	-1.9	-6.3
Chestnut	Control	7	0.0	0.00	0.0	0.0
Chestnut	Treatment	9	-21.5	27.05	1.6	-83.6
Chicory	Control	1	0.0	NA	0.0	0.0
Chicory	Treatment	1	-2.4	NA	-2.4	-2.4
Chitosan	Control	2	0.0	0.00	0.0	0.0
Chitosan	Treatment	4	2.3	21.10	33.9	-9.3
Chloroform	Control	1	0.0	NA	0.0	0.0
Chloroform	Treatment	1	38.0	NA	38.0	38.0
Coconut	Control	2	0.0	0.00	0.0	0.0
Coconut	Treatment	3	-26.7	60.35	14.4	-96.0
Coconut inclusion	Control	9	0.0	0.00	0.0	0.0
Coconut inclusion	Treatment	16	-57.2	68.93	-1.1	-211.0
Corn	Control	1	0.0	NA	0.0	0.0

Corn	Treatment	3	26.3	22.74	45.0	1.0
Cumin	Control	1	0.0	NA	0.0	0.0
Cumin	Treatment	2	-1.8	0.27	-1.6	-1.9
Cysteine	Control	4	0.0	0.00	0.0	0.0
Cysteine	Treatment	4	-3.5	11.67	8.6	-19.5
DDGS concentrate	Control	1	0.0	NA	0.0	0.0
DDGS concentrate	Treatment	3	-45.0	8.54	-37.0	-54.0
Defaunation	Control	2	0.0	0.00	0.0	0.0
Defaunation	Treatment	2	-1.3	0.51	-0.9	-1.6
DHA inclusion	Control	3	0.0	0.00	0.0	0.0
DHA inclusion	Treatment	5	9.6	21.38	35.0	-23.0
Essential oil blend	Control	4	0.0	0.00	0.0	0.0
Essential oil blend	Treatment	4	-14.8	16.58	3.8	-36.5
Eucalyptus	Control	1	0.0	NA	0.0	0.0
Eucalyptus	Treatment	1	-7.2	NA	-7.2	-7.2
Eugenol	Control	1	0.0	NA	0.0	0.0
Eugenol	Treatment	3	-15.7	2.52	-13.0	-18.0
Fatty acid blend inclusion	Control	4	0.0	0.00	0.0	0.0
Fatty acid blend inclusion	Treatment	9	-23.7	38.30	17.0	-84.0
Fibrolytic enzyme	Control	3	0.0	0.00	0.0	0.0
Fibrolytic enzyme	Treatment	4	27.0	35.22	74.0	-0.1
Flavomycin	Control	1	0.0	NA	0.0	0.0
Flavomycin	Treatment	1	-1.9	NA	-1.9	-1.9
Flavonoids	Control	1	0.0	NA	0.0	0.0
Flavonoids	Treatment	1	-2.2	NA	-2.2	-2.2
Flaxseed inclusion	Control	5	0.0	0.00	0.0	0.0
Flaxseed inclusion	Treatment	5	-11.6	31.31	24.0	-58.1
Fumaric acid	Control	13	0.0	0.00	0.0	0.0
Fumaric acid	Treatment	19	-5.7	9.46	11.3	-27.2
Garlic	Control	13	0.0	0.00	0.0	0.0
Garlic	Treatment	16	0.3	5.31	15.0	-7.7
Glycerin	Control	2	0.0	0.00	0.0	0.0
Glycerin	Treatment	6	0.6	5.00	9.9	-4.1
GOS	Control	12	0.0	0.00	0.0	0.0
GOS	Treatment	12	1.6	6.99	17.2	-8.3
Grape marc	Control	1	0.0	NA	0.0	0.0
Grape marc	Treatment	2	-88.0	9.90	-81.0	-95.0
Grass	Control	3	0.0	0.00	0.0	0.0
Grass	Treatment	3	0.5	1.79	2.5	-0.9
Hydrolysable tannins	Control	2	0.0	0.00	0.0	0.0
Hydrolysable tannins	Treatment	4	3.7	1.55	5.6	1.8
Isobutyrat inclusion	Control	1	0.0	NA	0.0	0.0

Isobutyrat inclusion	Treatment	3	-2.6	1.27	-1.2	-3.6
Isovalerate inclusion	Control	1	0.0	NA	0.0	0.0
Isovalerate inclusion	Treatment	3	-2.8	1.60	-1.0	-4.1
Lasolocid	Control	3	0.0	0.00	0.0	0.0
Lasolocid	Treatment	3	-0.2	5.38	5.0	-5.7
Lauric	Control	5	0.0	0.00	0.0	0.0
Lauric	Treatment	7	-20.3	63.05	81.0	-96.0
Leather strap	Control	1	0.0	NA	0.0	0.0
Leather strap	Treatment	3	-1.9	1.28	-1.1	-3.4
Legume	Control	5	0.0	0.00	0.0	0.0
Legume	Treatment	5	1.7	4.17	8.7	-2.5
Linoleic inclusion	Control	1	0.0	NA	0.0	0.0
Linoleic inclusion	Treatment	1	-2.5	NA	-2.5	-2.5
Linseed inclusion	Control	14	0.0	0.00	0.0	0.0
Linseed inclusion	Treatment	18	-40.7	55.95	23.0	-196.1
Lotus tannins	Control	3	0.0	0.00	0.0	0.0
Lotus tannins	Treatment	3	0.2	20.94	23.4	-17.4
Lovastatin	Control	2	0.0	0.00	0.0	0.0
Lovastatin	Treatment	4	13.8	13.25	29.1	0.4
Lupine seed	Control	3	0.0	0.00	0.0	0.0
Lupine seed	Treatment	3	-4.3	8.03	2.6	-13.1
Maca	Control	3	0.0	0.00	0.0	0.0
Maca	Treatment	3	-3.5	8.46	5.9	-10.5
Malic acid	Control	2	0.0	0.00	0.0	0.0
Malic acid	Treatment	3	-19.8	17.21	-5.2	-38.8
Methylbutyrate inclusion	Control	1	0.0	NA	0.0	0.0
Methylbutyrate inclusion	Treatment	3	-2.3	1.43	-0.7	-3.4
Mimosa	Control	2	0.0	0.00	0.0	0.0
Mimosa	Treatment	3	-2.5	3.23	0.8	-5.7
Monensin	Control	40	0.0	0.00	0.0	0.0
Monensin	Treatment	45	-14.9	23.32	27.3	-92.4
Monensin blend	Control	1	0.0	NA	0.0	0.0
Monensin blend	Treatment	3	-14.6	18.17	6.2	-27.4
Myristic acid	Control	3	0.0	0.00	0.0	0.0
Myristic acid	Treatment	4	11.8	36.35	63.0	-18.0
Myristic acid inclusion	Control	4	0.0	0.00	0.0	0.0
Myristic acid inclusion	Treatment	4	-81.6	85.88	-4.1	-156.0
Nisin	Control	2	0.0	0.00	0.0	0.0
Nisin	Treatment	2	-2.1	0.97	-1.4	-2.8
Nitrate	Control	35	0.0	0.00	0.0	0.0
Nitrate	Treatment	43	-39.5	34.65	4.0	-144.8
Nitrate and Sulfate	Control	1	0.0	NA	0.0	0.0

Nitrate and Sulfate	Treatment	1	-7.8	NA	-7.8	-7.8
Nitroethane	Control	2	0.0	0.00	0.0	0.0
Nitroethane	Treatment	5	-53.8	41.67	-28.6	-127.9
Oregano	Control	3	0.0	0.00	0.0	0.0
Oregano	Treatment	5	-127.7	120.56	-2.4	-298.0
Peppermint	Control	1	0.0	NA	0.0	0.0
Peppermint	Treatment	1	-27.5	NA	-27.5	-27.5
Polyethylene glycol	Control	4	0.0	0.00	0.0	0.0
Polyethylene glycol	Treatment	4	15.5	22.08	48.4	2.7
Propanediol	Control	1	0.0	NA	0.0	0.0
Propanediol	Treatment	1	-1.7	NA	-1.7	-1.7
Proteolytic enzyme	Control	1	0.0	NA	0.0	0.0
Proteolytic enzyme	Treatment	1	-9.3	NA	-9.3	-9.3
Quebracho	Control	2	0.0	0.00	0.0	0.0
Quebracho	Treatment	6	-15.7	19.68	3.0	-41.1
Rumen protected FA inclusion	Control	1	0.0	NA	0.0	0.0
Rumen protected FA inclusion	Treatment	1	-33.7	NA	-33.7	-33.7
Rumen protected fat	Control	1	0.0	NA	0.0	0.0
Rumen protected fat	Treatment	1	-1.4	NA	-1.4	-1.4
Saifoin maturity	Control	2	0.0	0.00	0.0	0.0
Saifoin maturity	Treatment	2	24.0	1.41	25.0	23.0
Saifoin tannins	Control	1	0.0	NA	0.0	0.0
Saifoin tannins	Treatment	5	-5.6	13.97	7.0	-23.0
Saponaria	Control	3	0.0	0.00	0.0	0.0
Saponaria	Treatment	3	-16.6	13.99	-4.5	-31.9
Sericea lespedeza tannins	Control	4	0.0	0.00	0.0	0.0
Sericea lespedeza tannins	Treatment	4	-3.2	1.01	-2.0	-4.1
Sodium bicarbonate	Control	1	0.0	NA	0.0	0.0
Sodium bicarbonate	Treatment	1	-3.3	NA	-3.3	-3.3
Sorghum tannins	Control	6	0.0	0.00	0.0	0.0
Sorghum tannins	Treatment	6	1.5	2.15	4.1	-0.9
Soybean oil inclusion	Control	2	0.0	0.00	0.0	0.0
Soybean oil inclusion	Treatment	2	-0.5	1.79	0.7	-1.8
Stearic	Control	5	0.0	0.00	0.0	0.0
Stearic	Treatment	7	-8.8	61.32	81.0	-96.0
Styzolobium tannins	Control	1	0.0	NA	0.0	0.0
Styzolobium tannins	Treatment	1	-0.1	NA	-0.1	-0.1
Sucrose	Control	1	0.0	NA	0.0	0.0
Sucrose	Treatment	1	0.0	NA	0.0	0.0
Sulfate	Control	1	0.0	NA	0.0	0.0
Sulfate	Treatment	1	-5.4	NA	-5.4	-5.4
Sulla tannins	Control	4	0.0	0.00	0.0	0.0

Sulla tannins	Treatment	4	-0.3	4.99	5.1	-6.1
Sunflower inclusion	Control	3	0.0	0.00	0.0	0.0
Sunflower inclusion	Treatment	4	-34.3	26.25	-1.7	-57.8
Sunphenon	Control	1	0.0	NA	0.0	0.0
Sunphenon	Treatment	3	-4.1	2.91	-1.8	-7.4
Tallow inclusion	Control	1	0.0	NA	0.0	0.0
Tallow inclusion	Treatment	1	-24.0	NA	-24.0	-24.0
Tea saponin	Control	6	0.0	0.00	0.0	0.0
Tea saponin	Treatment	6	-6.3	7.50	-0.7	-18.3
Triiodothyronine	Control	2	0.0	0.00	0.0	0.0
Triiodothyronine	Treatment	2	-0.7	1.94	0.7	-2.1
Valonea	Control	2	0.0	0.00	0.0	0.0
Valonea	Treatment	2	0.2	1.37	1.1	-0.8
Vitacogen	Control	2	0.0	0.00	0.0	0.0
Vitacogen	Treatment	2	5.2	9.63	12.0	-1.6
Yeast	Control	9	0.0	0.00	0.0	0.0
Yeast	Treatment	9	-9.9	16.77	7.2	-42.0
Yucca	Control	9	0.0	0.00	0.0	0.0
Yucca	Treatment	12	-2.0	4.81	6.7	-12.2

Appendix 2. Summary of manure additives investigated in this study.

Additives Type	Ingredient	Reference	Year	Species	Meta-analysis inclusion
Acidification	aluminum sulfate	Regueiro et al.	2016	pig	Yes
	calcium superphosphate	Zhang, et al.	2017	pig	No
	food industrial waste	Samer, et al.	2014	dairy	Yes
	hydrochloric acid	Petersen, et al.	2012	cattle	No
	lactic acid	Berg, et al.	2006	cattle	No
	methionine	Petersen, et al.	2012	cattle	No
	nitric acid	Berg, et al.	2006	cattle	No
	phosphogypsum	Hao, et al.	2005	cattle	No
		Luo, et al.	2013	pig	No
	sulfate	Petersen, et al.	2012	cattle	No
	sulfuric acid	Misselbrook, et al.	2016	cattle	No
		Owusu-Twum, et al	2017	cattle	No
		Wang, et al.	2014	pig	No
	wood vinegar	Wang, et al.	2018	pig	No
Adsorbent	zeolite	Wheeler, et al.	2010	dairy	No
		Wang, et al.	2018	pig	No
	clay	Chen, et al.	2018	chicken	No
Biochar	bamboo	Chen, et al.	2017	hen	No
		Liu, et al.	2017	hen	No
		He, et al.	2019	pig	No
	charcoal	Chowdhury, et al.	2014	hen	Yes
	coir	Chen, et al.	2017	hen	No
	cornstalk	Chen, et al.	2017	hen	No
	greenwaste	Agyarko-Mintah, et al.	2017	poultry	Yes
	layer manure	Chen, et al.	2017	hen	No
	poultry litter	Agyarko-Mintah, et al.	2017	poultry	Yes
	rice hull	Jia, et al.	2016	chicken	No
	rice straw	He, et al.	2019	pig	No
	woody	Chen, et al.	2017	hen	No
	N/A	Vandecasteele	2016	chicken	Yes
	N/A	Sonoki, et al	2011	cattle	No
	N/A	Mao, et al.	2018	pig	No
	N/A	Wang, et al.	2018	pig	No
	N/A	Chowdhury, et al.	2014	animal	No
Biological materials	EU200	Owusu-Twum, et al	2017	cattle	No
	Biobuster	Owusu-Twum, et al	2017	cattle	No
	Biosuper	Martinez, et al.	2003	pig	No

C/N content	sawdust	Jia, et al.	2016	chicken	No
	plastic tube pieces	Chowdhury, et al.	2014	animal	No
	woodchips	Chowdhury, et al.	2014	animal	No
	lupin residues	Chowdhury, et al.	2014	animal	No
Disinfection	sodium tetraborate decahydrate	Wheeler, et al.	2010	dairy	No
	hydrogen peroxide	Wheeler, et al.	2010	dairy	No
	oxychlorine solution	Wheeler, et al.	2010	dairy	No
Essential oil	carvacrol and pinene	Wheeler, et al.	2010	dairy	No
	eugenol	Wheeler, et al.	2010	dairy	No
	glycerol	Wheeler, et al.	2010	dairy	No
	basil	Wheeler, et al.	2010	dairy	No
	peppermint black mitchium	Wheeler, et al.	2010	dairy	No
	hyssopus oil	Wheeler, et al.	2010	dairy	No
Humate	ManureMax	Shah, et al.	2012	swine	No
Microbial digestion	aerobic/facultative microbes	Wheeler, et al.	2010	dairy	No
	mixture of chemicals and surfactants for facultative bacteria	Wheeler, et al.	2010	dairy	No
	aerobic/facultative microbes with growth factors	Wheeler, et al.	2010	dairy	No
	aerobic microorganism	Mao, et al.	2018	pig	No
	facultative microorganisms	Mao, et al.	2018	pig	No
Other chemical	Stalosan	Martinez, et al.	2003	pig	No
	NX23	Martinez, et al.	2003	pig	No
Oxidizing agent	mixture chemicals/micronutrient concentrate	Wheeler, et al.	2010	dairy	No
	mixture of chemicals in isopropyl alcohol	Wheeler, et al.	2010	dairy	No
	mixture of chemicals	Wheeler, et al.	2010	dairy	No
	complex triazine mixture	Wheeler, et al.	2010	dairy	No
	Abandoned mine drainage	Wheeler, et al.	2010	dairy	No
	dipole dibase formulation	Wheeler, et al.	2010	dairy	No
Physical agent	sand	Hao, et al.	2005	cattle	No
Straw	N/A	Yamulki	2006	cattle	Yes
	barley straw	Sommer, et al.	2000	pig	No
		Chowdhury, et al.	2014	animal	No

The Potential for Biochar to Enhance Sustainability in the Dairy Industry

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EXECUTIVE SUMMARY

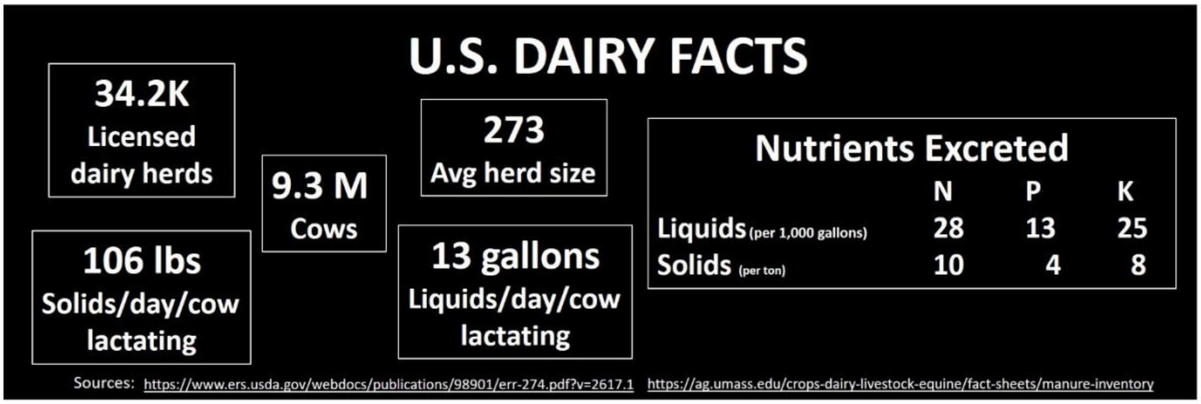
The US dairy industry launched a net zero initiative with the objective of becoming carbon neutral or even carbon negative by 2050 along with adoption of goals to optimize water use and improve water quality by recycling manure-based nutrients in dairy farms (ICUSD, 2020). Reducing impacts on air and water quality, and greenhouse gas (GHG) emissions such as methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2) is increasingly urgent. Regulations are increasingly causing farmers to build larger facilities or buy more land to handle excess nutrients. At the same time, the industry has been hit hard by a number of different challenges.

New methods of reducing emissions on dairy farms include the production and use of biochar, a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment. Biochar can be used as a product itself or as an ingredient within a blended product, to improve soil properties and/or resource use efficiency, to remediate and/or protect against environmental pollution, and as an avenue for GHG mitigation (IBI 2013). Biochar also offers the possibility of large-scale carbon sequestration which may lead to increased revenues for farmers of all types as carbon marketplaces begin to embrace biochar as a carbon removal product.

This paper reviews some ways that biochar is being or could be incorporated on dairy farms to improve overall economic and environmental impacts. While additional benefits and uses could accrue to the entire supply chain for milk, cheese any other milk-based products, this paper focuses solely on the dairy farm itself. It reviews different entry points for biochar from its use as a feed additive, to feed storage component, bedding additive, or manure management component. It also discussed converting manure directly into biochar as a manure management strategy that could reduce storage costs and GHG emissions.

The methodology used in this paper combines a review of the peer-reviewed literature with a survey of selected dairy and biochar demonstration projects in Australia, Canada, and the United States. Several project teams were interviewed, and project descriptions are included which outline preliminary results of using biochar within the context of dairy farming.

While a growing number of dairies are discovering the benefits of biochar, much work remains to help scale the production and use of biochar within the industry. Recommendations for future activities include benchmarking the GHG reductions for Thermochemical Conversion (TCC) compared to different manure management processes, optimizing TCC technologies for different sized dairies and those with existing infrastructure for manure management (e.g. anaerobic digesters), additional research on the impact of adding biochar to dairy feed on milk production, more biochar production demonstrations on dairy farms, and on-going coordination amongst dairy and biochar projects.



CHALLENGING TIMES FOR DAIRIES

Dairy farmers have faced many challenges particularly over the last decade. Milk prices are falling (Reese 2019); equipment costs are on the rise, with some essential farm equipment more than doubling in cost since 1995 (Koenig 2016); and availability of labor for the dairy industry has steadily decreased (USDA "Farm Labor"). Although the average number of milking cows decreased in 2019, US milk productivity has more than doubled over the past several decades (Blayney 2004) resulting in more supply than demand leading to falling prices and losses that averaged $3 per hundredweight of milk produced in 2018 (Mercier 2019). This is an unsustainable situation which has forced an increasing number of dairies out of the industry.

Farmers are also facing increasing scrutiny about the environmental impacts of nutrients in land-applied dairy manure that can impact local and regional water bodies (Eagle 2017). For instance, the public often blames phosphorus runoff from dairy farms as the primary cause of harmful algal blooms (Guo et al. 2019).

USES & BENEFITS OF BIOCHAR ON DAIRY FARMS

Biochar, at its most basic, is carbonized organic material. It can be produced using a wide variety of thermochemical conversion technologies, and from a wide variety of feedstocks including wood, crop residues, and manure. Although most often biochar is intended for direct use in soils as a soil amendment to improve soil health and to reduce land degradation, additional benefits may accrue for the dairy industry both on-farm and for surrounding communities. These benefits are outlined below.

Feed Additive

Although the use of biochar as a feed additive for animals that enter the human food chain was removed from the Food & Drug Administration list of approved additives in the United States roughly a decade ago, at least one State has approved of its use for livestock. The California Department of Food & Agriculture allows the use of biochar (called charcoal in their regulations) in livestock feed. The Official California Code of Regulations for Food and Agriculture related to commercial feed states the following:

> *(e) Charcoal (vegetable) is charred hard or soft wood, nut shells, or fruit pits. If it is wood charcoal, it shall bear a designation indicating whether it is hard wood charcoal or soft wood charcoal. Charcoal from nut shells or fruit pits shall be designated as shell charcoal. When used in a mixed feed the maximum percent shall be stated on the label. (Barclay's Official California Code of Regulations)*

Feed biochar has been approved and used in many other parts of the world for many years including Europe, Australia, Canada, and Japan (Schmidt et al. 2019). In some of these areas, feed is a larger market for biochar than the soil amendment market. Significant interest and attention within the US biochar industry is focused on expanding biochar to the list of federally approved feed additives. In Europe, the certification criteria for feed biochar is more stringent than for soil use biochar. Currently biochar used as a feed additive in Europe is limited to biochar made using woody material only (EBC 2012).

Both academic and anecdotal studies are increasingly demonstrating the benefits that can be derived from adding small amounts of biochar to animal feed. Schmidt et al. (2019) summarized research on the use of biochar as a feed additive and found it offers the following benefits: improved animal health, increased feed efficiency and healthier atmosphere for animals, reduced nutrient losses and greenhouse gas emissions, and once the manure is applied to soils, increased soil organic matter content and soil fertility. While some studies suggest that using biochar as a feed additive may reduce enteric methane emissions from ruminants (Lang et al. 2015, Winders et al. 2019), others suggest negligible or no reduction of methane emissions (Teoh et al. 2019, Terry et al. 2019). Ultimately, this is an area of research that needs more standardized methods and further investigation (Kammann et al. 2017). Activated carbon, which is similar to biochar but undergoes more extensive processing and is often more expensive, acts as a binder when fed to livestock and has been shown to reduce certain mycotoxins that may be found in animal feed that contaminate milk and meat. Up to 93% removal efficiencies of aflatoxin in milk have been observed when high surface area activated carbons are added to dairy feed. In the same study bentonite, a commonly used binder, removed 80% of the aflatoxins (Di Natale et al. 2009).

Doug Pow, a cattle and avocado farmer in Western Australia, has been feeding his cows biochar mixed with molasses for the past seven years (IBI 2018). He has collaborated extensively with academic researchers who have studied and reported on his methods and outcomes. Originally, his goal was to add long lasting organic matter to his pastures by employing his cows as a low-cost delivery system in collaboration with dung beetles that would carry the biochar enriched dung further down into the soil profile. His pastures have become much more fertile while eliminating the need to purchase fertilizer or additional hay for feed. At the same time his cattle have become healthier even as he reduced or eliminated the use of insect sprays and drenches (Joseph et al. 2015).

Feed Storage

Storing large quantities of silage can generate leachate that contains biological oxygen demand (BOD) in the range of 12,000 – 90,000 mgL^{-1} (Sandford et al. 2020). As a point of comparison, the BOD from various wastewater treatment plants in the United States ranged from 101 – 437 mgL^{-1} (Sieple et al. 2017). Without proper controls nitrogen (N), phosphorus (P), and potassium (K) from silage runoff can contaminate groundwater or nearby water bodies. While the leachate can be added to manure storage facilities, this can produce dangerous hydrogen sulfide (H_2S), and must therefore be managed carefully.

One approach which is sometimes used to manage seepage is vegetated filter strips. Adding 2.5% (wt/wt) corncob biochar to a depth of 15 cm in vegetated filter strips surrounding horizontal bunker silos has been shown to reduce cumulative total nitrogen (TN) influent by 64% whereas the control reduced TN by 49%. While vegetative strips can reduce cumulative nitrate (NO_3^-) leaching, the addition of biochar reduced it by an additional 40% (Sandford et al. 2020). Once the biochar in the filter strips becomes saturated with these nutrients, it could be removed and applied to soils as a source of nutrients reducing the need to purchase additional fertilizers.

Bedding Additive

Few rigorous scientific studies have been published comparing the use of biochar in dairy or other livestock bedding to current bedding options and inputs (e.g. sand, sawdust, lime, gypsum, etc.). However, an increasing number of on-farm experimentation has shown that using biochar as a component of bedding could lead to numerous benefits including reduced odors, dryer stalls and improved hoof health.

An inoculated deep litter system (IDLS) developed as part of the Korean Natural Farming program includes a 6” layer of biochar at the bottom of the system, covered with deep layers of logs and green waste. Adding micro-organisms to this type of bedding results in lower odor, fewer flies and can significantly reduce labor related to cleaning as the systems can last for 10 years or more, according to Mike DuPonte, an Animal Specialist with CTHAR Cooperative Extension in Hawaii (DuPonte et al. 2012).

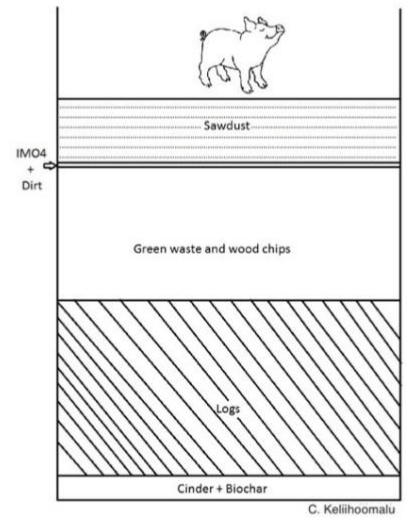


Diagram showing layers in IDLS pen.

MANURE MANAGEMENT

Dairy cows produce prodigious amounts of manure; daily manure production ranges between 52 to 67 kg per animal per day. Manure management for even small-scale dairies can thus be challenging, especially if there is insufficient land for spreading manure, or if regulations and weather prohibit spreading during certain months of the year.

Manure management strategies vary. Some of the most common include composting, slurries or lagoons, and anaerobic digestion for larger dairies. Biochar can be added to composting, slurries or lagoons, or manure can be converted directly into biochar via thermo-chemical conversion. Each of these are manure management strategies are discussed below.

Land Application of Manure

Dairy farmers have commonly applied manure or slurry to their land as a way to recycle the nutrients. However, this can lead to excess nutrient leaching, particularly of P, into nearby waterbodies resulting in eutrophication or harmful algal blooms (HABs) (Carpenter et al. 1998).

Emissions from land application of manure can also be significant (FAO 2010). Brennan et al. (2015) compared the impact of different types of slurry on emissions when being applied to land. The addition of biochar made from wood shavings pyrolyzed at 650°C for 4.5 hours significantly reduced nitrous oxide (N_2O), ammonia (NH_3), cumulative carbon dioxide (CO_2) (by 63%, 72%, and 84% respectively) and thereby reduced overall Global Warming Potential (Forster et al. 2007) of land application of dairy cattle manure.

When comparing direct land application of cattle manure to gasification of manure followed by land application, emissions varied from a positive emissions rate of 119 kg to negative emissions of -643 per ton of dry manure (Wu et al. 2013). This calculation assumes energy from the gasification process will be used to displace fossil fuel energy.

Composting

Composting dairy manure is a common manure management strategy though it has certain limitations in terms of pathogen removal and environmental issues, such as greenhouse gas (GHG) emissions and odors. The use of biochar in manure composting offers several potential advantages. Co-composting of up to 10% biochar by dry weight with manure or other organic material can provide several benefits including increased nutrient retention, reduced emissions of NH_3, N_2O and methane (CH_4), reduced bioavailability of heavy metals (e.g. copper (Cu), cadmium (Cd), and zinc (Zn)), improved water management and aeration and odor reduction (Sanchez et al. 2018). In addition, biochar provides a habitat for various microorganisms which enhance the composting process.

Biochar has been found to accelerate and improve the composting process when added at the beginning—for example by increasing temperature which stimulates microbial activity. This increased activity and higher temperatures can also reduce certain pathogens. Biochar addition has been shown in both research and commercial operations to reduce labor for turning piles and improve habitat for microorganisms, enhance moisture, aeration and nutrient availability thereby boosting microbial growth (Sanchez et al. 2018). This may have important economic implications since accelerated composting is a desirable effect, especially with organic materials that require long composting times and take up space.

Biochar addition to compost has been found to reduce emissions of N_2O, which result from the animal manure composting process (Akdeniz 2019), by 26% (Wang et al. 2013). Adding biochar to compost has also proven useful in reducing CH_4 emissions (Pandey et al. 2014, Chen et al. 2017). For instance, researchers at the University of Merced in California are investigating the impact biochar has on CH_4 from dairy manure and compost. They have hypothesized that it could reduce state-wide CH_4 emissions related to manure by 2.75 Mt CO_2e per year (Feedstuff.com 2019).

Biochar additions during the composting process can also reduce NH_3 gas losses by between 50 – 64% (Steiner et al. 2010; Malinska et al. 2014; Agyarko-Mintah et al. 2017*)* which can cause nuisance odors and is a major source of N loss (Eghball et al. 1997; Bernal et al. 2009). The ammonia gas retention can be enhanced with greater oxidation of the biochar (Hestrin et al. 2019).

Slurry/Lagoon

NH_3 emissions from manure slurries can cause various environmental problems, including poor air quality. After heavy precipitation events slurries may get overloaded and leak N, leading to eutrophication and algae blooms. Biochar used as a floating manure cover on slurries or lagoons can significantly reduce NH_3 emissions. Holly et al. (2017) found that woody biochar produced from low temperature pyrolysis (400°C) was able to reduce NH_3 emissions by 96% as compared to an uncovered slurry. Layering biochar on top of the slurry creates a barrier that reduces volatilization and related odors.

Daugherty et al. (2017) found that biochar made from bark and center wood pyrolyzed at 600°C could reduce NH_3 concentrations in head space between 72 – 80%, yet biochar made by gasifying Douglas Fir at 600°C did not significantly impact ammonia concentrations and associated odors, biochar covers can sorb nutrients such as N and P. Information on CH_4 emissions from lagoons to which biochar was added, are currently lacing and require further research.

Anaerobic Digestion

Larger dairy farms may use an anaerobic digester (AD) which is an oxygen-free environment that converts the organic material into CH_4, CO_2, and H_2S. While there are many benefits to AD systems, they can be expensive to install and maintain. The process provides renewable energy but does not significantly reduce the volume of material and farmers must still have storage facilities or markets for the fiber (digestate) and/or sufficient land to spread the nutrient-rich effluent.

Co-locating pyrolysis with AD may be able to offer a synergistic manure management system. Adding 10 g L^{-1} of biochar made from dairy manure pyrolyzed at 350°C was found to increase methane production by 25% while decreasing the lag phase from 2 days to 1.5 days (Jang et al. 2020).

Additional benefits of using biochar in an AD include a substantial reduction of H_2S production that could lead to improved biogas quality and reduced wear and tear on equipment. Wang (2018) observed a 78% reduction of H_2S using poplar woodchip biochar while Choudhury & Lansing (2019) found that iron (Fe) impregnated biochar had a 99% removal efficiency for H_2S.

Thermo-Chemical Conversion

Thermo-chemical conversion (TCC) are high-heat, low- or no-oxygen processes that convert organic matter into gases, liquids and solids, including biochar, a material that decomposes much more slowly than the original biomass. There are various technologies capable of carbonizing organic material, but the most common are pyrolysis and gasification (hydrothermal carbonization produces hydrochar and is not considered in this white paper). TCC has a number of advantages over other manure management processes including volume reduction, faster processing, heavy metal immobilization, and the ability to reduce certain toxins and odors in the material that is converted.

Larger livestock operations may increasingly be required to provide manure storage capable of safely containing significant amounts of manure during certain times of the year when regulations do not permit manure application to soil. Even large manure storage can be threatened by large amounts of rainfall causing spillage. Volume reduction between 75% to 95% of the separated solids of the manure can be achieved using thermo-chemical conversion, depending on the temperature used for pyrolysis.

While other manure management processes such as composting or AD may take several weeks to process per batch, TCC converts manure into more persistent carbon in seconds to hours depending on the technology and desired co-products (typically about 15-30 minutes for slow pyrolysis). A continuous TCC process can reduce the need for expensive manure management infrastructure.

Current manure management strategies may be hotspots for certain contaminants such as antibiotics. Pyrolysis (>400°C) is capable of eliminating antibiotics and immobilizing heavy metals such as zinc, copper, chromium, nickel, lead and cadmium that are sometimes found in manure, can accumulate in soil, and negatively impact soil fertility and food safety (Tien et al. 2019; Li et al. 2019). Carbonizing manure also reduces the risk of spillage and overflow of storage systems during storms, if the need for storage is reduced.

Processing manure through TCC may also help reduce or eliminate certain pathogens, particularly those that are susceptible to high heat such as *E. coli* and salmonella.

Under certain conditions and with certain types of organic materials and thermochemical conversion technologies, pollutants such as polycyclic aromatic hydrocarbons (PAHs) or dioxins may be produced (Hale et al. 2012). However, the amount of these toxins is typically below regulated levels and in a majority of biochars tested, these contaminants were tightly bound and only marginal bioavailability (Hilber et al. 2017). Biochar may actually be able to reduce the availability of PAHs found in other materials such as sewage sludge (Stefaniuk et al. 2018).

DAIRY WASTEWATER

The amount of water consumed within the dairy industry is estimated to be 2.5 times the volume of milk produced and is considered one of the largest generators of industrial food wastewater in the world (Kolev Slavov 2017). Farm dairy effluent results from cleaning, disinfection of equipment, cooling and heating and contains water, urine, dung, feed, cleaning chemicals and milk. While the dry matter content is generally very low, dairy effluent contains nutrients such as N, P, K, and other elements. Though these nutrients can be beneficial in soils, but they can also lead to groundwater pollution which has motivated many local and state authorities to restrict the timing and amount of land spreading of dairy effluent. Ghezzehei et al. (2014) found that low temperature hardwood biochar added to the wastewater can retain 20% to 43% of ammonium (NH_4^+) and 19 - 65% phosphate (PO_4^{3-}) (2.86 mg and 0.23 mg per gram of biochar, respectively) over a 24-hour period. Biochar made from digestate was found to sorb up to 32% of P from anaerobically digested dairy effluent which was predominantly plant available and filtered through the biochar (Streubel 2011). Other research has demonstrated that biochar used in a filtration system can significantly reduce total suspended solids and the chemical oxygen demand by 85% and 83%, respectively (Samkutty & Gough 2002). Information about biochar used as a cover for dairy waste water is not available.

ENERGY PRODUCTION

TCC technologies capable of creating biochar include pyrolysis and gasification. Pyrolysis thermally decomposes biomass without the presence of oxygen to create biochar at temperatures starting at 300°C. Gasification uses limited oxygen and higher temperatures (500°C to 1,500°C) (Brown et al. 2015). A co-product of biochar production is energy in the form of process heat, liquid fuel, or combustible gases that can be used to supply heat or electricity. Depending on the technology used, additional co-products of TCC may include syngas and bio-oil in addition to biochar and heat. Often when high moisture content materials, such as manure or sewage are used for biochar production, this heat is used to dry organic materials prior to carbonization.

BIOCHAR FROM DAIRY RESIDUES

Table 1 highlights a number of papers that analyzed various characteristics of manure-derived biochar.

A recent study in 2018 as part of a project funded by the Innovation Center for US Dairy (Enders et al. 2019) analyzed pyrolyzed dairy manure in New York State. They found the pH of the biochar produced from dairy manure to be 10.4. More importantly, it had a calcium carbonate equivalence of 3.3%. In other words, 100 pounds of the manure biochar could neutralize acid as well as 3.3 pounds of lime. The organic carbon content of the biochar derived from dairy manure was 43%, and the quality of the carbon in the biochar is such that roughly half is expected to persist over 100 years, compared to practically 0% in the original manure.

The Fertilizer Class of the dairy manure biochar, according to the IBI classification system, was 3 on a scale of 0-4. This is defined as providing adequate nutrition for corn at <4.5 tons/acre for 3 out of 4 nutrients (Figure 1).

As for nutrients, Enders et al. (2019) found that the dairy manure biochar contained 4.1% phosphorus, 2.2% potassium, and 4.4% magnesium (Table 1). They found that nutrient concentration in the biochar could be as much 2.6 times greater than in the original manure feedstock and that sulfur in the biochar was 50% less than in the feedstock (Table 2). In addition to increasing total nutrient contents, pyrolysis improved nutrient availability. For instance, the biochar provided 13% more plant-available phosphorus (per unit total P) than the manure feedstock. Interestingly, increased available phosphorus was coupled with a 10-fold decrease in leachable phosphorus (i.e. the plant available phosphorus was not water soluble). The biochar also demonstrated 59% more available potassium than the manure.

Table 1. Characteristics of biochar from pyrolyzed Dairy Manure, Digested Dairy Manure, Composted Dairy Manure, Raw Dairy Manure, or Cow Manure (unspecified if it was dairy) from several sources.

Feedstock	Temperature (℃)	Total Ash Content (%)	Total C (%)	Total N (%)	H (%)	H:C ratio (mol:mol)	pH	Zinc (mg/kg)	Sodium (mg/kg)	Total P (mg/kg)	Calcium (mg/kg)	Magnesium (mg/kg)	Potassium (mg/kg)	Iron (mg/kg)	*Source*
Dairy Manure	0	14.80	46.52	2.29	5.49	1.41	8.30	220	2510	5610	16000	6940	6700	2290	*Cantrell et al. 2012*
Dairy Manure	300	10.10	61.50	1.60	4.50	0.87	-	90	3270	1152	11094	3934	8986	208	*Enders et al. 2012*
Dairy Manure	350	10.20	64.10	1.80	4.10	0.76	-	98	3698	1810	10859	4278	10074	317	*Enders et al. 2012*
Dairy Manure	350	-	42.85	2.36	-	-	9.72	150	1040	5730	33700	6510	5030	8290	*Liu et al.2014*
Dairy Manure	350	24.20	55.80	2.60	4.29	0.92	9.20	361	5620	10000	26700	1220	14300	3640	*Cantrell et al. 2013*
Dairy Manure	400	11.50	67.10	1.40	3.30	0.59	-	87	3569	1466	12808	4258	10345	305	*Enders et al. 2012*
Dairy Manure	450	11.70	70.10	1.50	3.10	0.53	-	121	4009	2001	13473	5068	11756	349	*Enders et al. 2012*
Dairy Manure	500	12.40	72.50	1.40	2.60	0.43	-	80	2223	1754	12569	4610	9630	396	*Enders et al. 2012*
Dairy Manure	500	-	73.87	1.38	2.42	0.39	10.18	-	-	-	-	-	-	-	*Ouyang et al. 2013*
Dairy Manure	500	-	44.67	1.98	-	-	10.20	170	1170	6460	38000	7340	5670	9340	*Liu et al.2014*
Dairy Manure	550	13.40	72.30	1.50	2.30	0.38	-	142	4424	2358	25702	6357	13388	754	*Enders et al. 2012*
Dairy Manure	600	12.60	75.20	1.30	2.00	0.32	-	114	4538	2433	13997	5366	13236	398	*Enders et al. 2012*
Dairy Manure	700	39.50	56.67	1.51	0.94	0.20	9.90	423	8790	16900	44800	2060	23100	6480	*Cantrell et al. 2014*
Digested Dairy Manure	300	39.20	56.10	2.70	-	-	9.00	129	3808	5391	20185	8757	14954	1710	*Enders et al. 2012*
Digested Dairy Manure	350	12.70	57.70	2.40	-	-	9.20	-	-	-	-	-	-	-	*Enders et al. 2012*
Digested Dairy Manure	400	14.50	63.80	2.40	-	-	9.30	131	4405	6446	22552	9733	16604	1656	*Enders et al. 2012*
Digested Dairy Manure	450	17.80	60.40	2.50	-	-	10.20	-	-	-	-	-	-	-	*Enders et al. 2012*
Digested Dairy Manure	500	14.70	59.40	2.60	-	-	9.70	224	3861	5649	18505	8498	14937	2371	*Enders et al. 2012*
Digested Dairy Manure	550	17.30	60.90	2.20	-	-	10.00	-	-	-	-	-	-	-	*Enders et al. 2012*
Digested Dairy Manure	600	18.80	62.80	2.20	-	-	10.00	200	5051	8269	26518	11744	20852	2356	*Enders et al. 2012*
Composted Dairy Manure	500	50.10	37.80	2.00	-	-	10.30	172	1219	6011	38388	12534	12824	9119	*Enders et al. 2012*
Raw Dairy Manure	500	32.00	51.20	2.10	-	-	10.70								*Enders et al. 2012*
Cow Manure	450	-	29.50	1.39	0.95	0.38	-	-	-	-	-	-	-	-	*Sun et al. 2013*
Cow Manure	600	-	30.70	1.11	0.46	0.18	-	-	-	-	-	-	-	-	*Sun et al. 2013*
Cow Manure	500	67.50	43.70	-	-	-	10.20	52	-	646	3795	1569	1021	616	*Zhao et al. 2013*
Cow Manure	500	-	43.70	1.99	3.20	0.87	-	-	-	-	-	-	-	-	*Zhao et al. 2014*

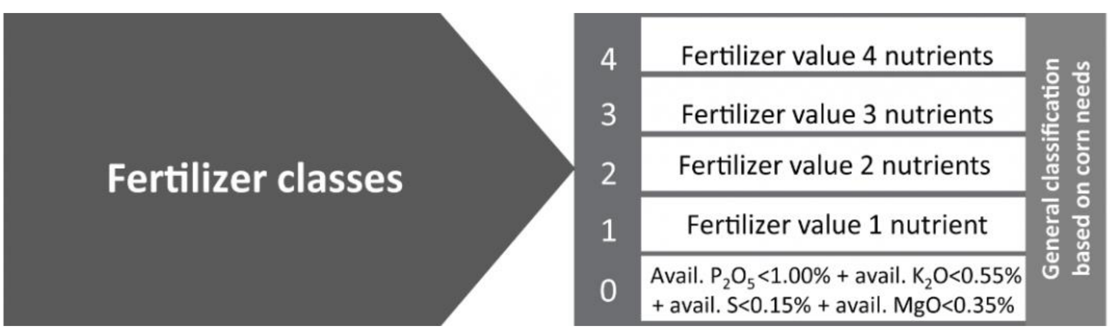


Figure 1. Fertilizer class based on the ability of P, K, S and Mg in a biochar to satisfy the expected yield and nutrient removal demands of corn. Courtesy of International Biochar Initiative https://biochar-international.org/biochar-classification-tool/

Table 2. Total nutrient contents and retention (i.e., the amount retained in the biochar as compared to the total amount in the original manure; full recovery would be 100%) of nutrients in uncharred manure and biochar made from the same manure (from Enders et al. 2019).

Element	Total content		Change due to pyrolysis	
	Manure (mg/kg)	Biochar (mg/kg)	Concentration	Retention
Phosphorous	10481.6	17728.6	69%	64%
Potassium	15721.5	21897.7	39%	53%
Calcium	154454.9	270710.8	75%	67%
Magnesium	15134.9	26391.3	74%	66%
Sulfur	8346.0	4212.6	-50%	19%
Iron	1801.3	4057.9	125%	86%
Manganese	214.3	369.7	72%	66%
Zinc	266.6	526.5	97%	75%

Enders et al. (2019) also did a stringent IBI toxicant assessment and found the dairy manure biochar did not contain toxic levels of any investigated compounds and contained 30 times less than the threshold value for any single analyte. A germination trial also assessed possible biochar toxicity. Of the three species used (lettuce, ryegrass, and radish) germination in dairy manure biochar amended media was not different from the control.

Using high temperature pyrolysis (900°C), it is possible to design dairy manure biochar with high surface area (360 m^2 g^{-1}) and high cation exchange capacity (57.5 ± 16.1 cmol kg^{-1}) (Tsi et al. 2019) (Figure 2). This type of biochar may be a cost-effective way to remove pollutants. As with activated carbons, dairy manure biochar could be regenerated and reused for extracting heavy metals such as lead (Pb), Zn, and Cd (Wallace et al. 2019).

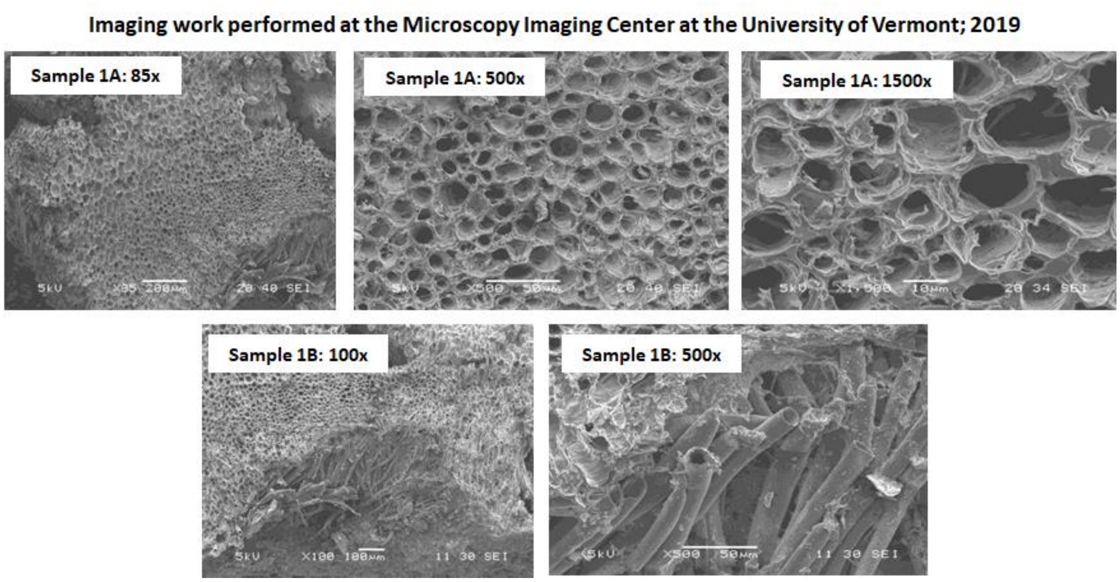


Figure 2. Dairy manure biochar samples at different magnification levels highlighting different pore sizes and surface areas.

ROLE OF BIOCHAR IN DAIRY OPERATIONS FOR ADAPTATION TO AND MITIGATION OF CLIMATE CHANGE

In many farming scenarios the production and use of biochar can help farmers to both adapt to the impacts of climate change and reduce their emissions that contribute to climate change. Both adaptation and mitigation are considered below.

Adaptation

While climate change impacts vary significantly by region, many areas are experiencing increasing drought while others must cope with heavier rainfall and higher temperatures. For the dairy industry, higher temperatures can lead to heat stress that reduces feed intake and milk production. Pasture production and crop yields are increasingly variable and the need for improved water efficiency is becoming critical in dryer regions. Adding certain types of biochar, either on their own or, preferably in combination with manure, to pastures and crop land can improve the water retention in soils boosting resilience against drought (Rasa et al. 2018, Sanchez-Garcia et al. 2019; Razzaghi et al. 2020). Similarly, infiltration after rainfall events can be increased through biochar additions depending on biochar and soil properties (Wang et al. 2016b; Wang et al. 2017). It should be noted that the specific impact on plant available water holding capacity is highly impacted by both type of biochar and type of soils (Amonette et al. 2019). Wood shavings biochar has also been shown to improve the infiltration rate during simulated heavy rain events while also reducing soil erosion in arid or semi-arid climates (Abrol et al. 2016).

Mitigation

Dairy farmers are not only impacted by climate change, but they contribute to it through GHG emissions and have the opportunity to contribute to atmospheric carbon dioxide reductions through soil carbon sequestration. The sources and amount of emissions vary widely depending on various farmer practices. For instance, according to a study comparing eight organic dairies with eight conventional dairies in Germany, an organic dairy farm emits on average 995 g per kg of Energy Corrected Milk (ECM) while a conventional farm emits 1,048 g per ECM (Frank et al. 2019). The largest proportion of emission sources stem from enteric methane while the largest difference in emissions amongst dairy and conventional farms is related to carbon sequestration in soil. Organic farms achieved a net sequestration rate of (-57 g CO_2-eq (kg ECM)$^{-1}$) while conventional dairies produced 82 g CO_2-eq (kg ECM)$^{-1}$). Frank et al. (2019) concluded that GHG reduction plans require farm specific strategies based on current emission sources.

As discussed previously in this White Paper, the production and use of biochar could help to reduce GHG emissions and sequester carbon in a variety of ways, typically 0.5-1.5 t CO_2-e t^{-1} dry manure for slow pyrolysis (Cowie et al. 2015). A significant amount of emissions comes directly from cows in the form of enteric emissions (i.e. belching). Understanding how to reduce these emissions by changing diets or incorporating effective feed additives is critical. In addition to the benefits described previously in the Feed Additive section, preliminary research has shown that certain types of feed biochar can reduce enteric methane emissions in cattle by up to 18% as measured by dry matter intake (Winders et al. 2019). This may vary depending on the type of biochar, the feeding regimen and possibly the breed of dairy cow.

Biochar added to soils either directly or indirectly after having passed through the rumen, adds carbon to the soil that will persist for longer periods of time than manure alone (decades to millennia). In addition to direct carbon sequestration, biochar may indirectly improve carbon storage in soils through negative priming. This additional indirect sequestration could contribute nearly as much carbon as is contained in the biochar itself (Blanco-Canqui et al. 2019), but varies strongly with soil and biochar type, with reductions across studies lying around 4% (Wang et al. 2016a).

ECONOMICS OF BIOCHAR USE IN DAIRY FARMING

The financial impact of biochar use on dairies is heavily dependent on how and why it is used. As an example, a recent study in South Australia funded by the Dairy Industry Fund and carried out by the Climate and Agricultural Support Group found that a dairy with 250 cows netted AUS$71,000 in additional profits from increased milk production and reduced feed costs after the cost of biochar was deducted. If credits for carbon sequestration were added for the excreted carbon in the biochar or for reductions in GHG emissions from the soil, additional revenues would accrue.

Few scientific studies have been done to assess the cost impact of converting manure into biochar as compared to other manure management strategies. As pyrolysis can be done on a continuous basis and the reductions in volume are significant, smaller manure storage facilities would be required. Manure storage facilities can be very costly and can emit GHG emissions that may not currently be controlled by regulation but might be in the foreseeable future. Thus, carbonizing manure could save dairy farmers from needing to invest in larger facilities as well as avoiding carbon penalties.

Farmers may want to utilize the biochar on-farm for different uses, which have been described previously. Alternatively, some farmers, particularly larger dairies, may have excess biochar that could be sold.

According to Enders et al. (2019) the nutrient value of the biochar as a substitute for other organic fertilizers could equate to $240-340/ton. Analyses suggest that over half of the carbon in the resulting biochar will persist over the long term, to benefit soil fertility and carbon sequestration for over a century after application. Dairy manure biochar is an odor- and pathogen-free, nutrient-rich soil conditioner with approximately twice the nutrient content of the original manure by mass, and more than three times that by volume. A study by Krounbi et al. (2019) suggests there may be a significant market value for biochar produced from high moisture content waste products compared to compost. Additional economic values should be seen on farms with the use of biochar as an additive to bedding, manure pits, soil, and more. There is also an economic benefit in the reduction of storage, transportation and spreading costs.

DAIRY AND BIOCHAR DEMONSTRATION PROJECTS

Australia: Fleurieu Peninsula

Main Focus: Feed additive

Dairy farmers in the Fleurieu Peninsula in South Australia have found feeding biochar to their dairy cows not only improves milk production, but also improves feed conversion reducing the amount of feed farmers need to purchase or grow. Feeding wood-based biochar at a rate of 150 grams per day, increased daily milk production by 1.4 liters per head (McCallum 2020).

Canada: Poelman-Murray Ltd, Ontario

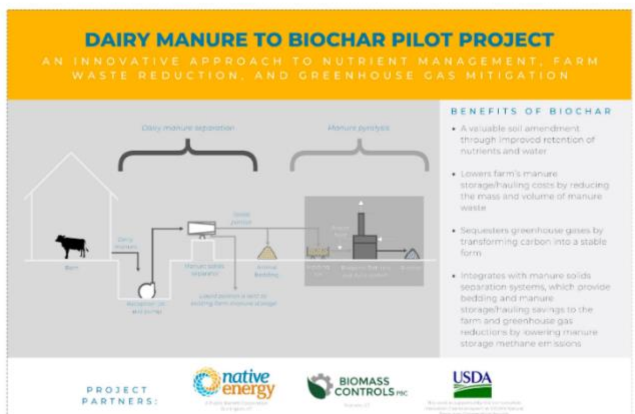
Main Focus: Feed additive

Holstein dairy farmer Thomas Murray began adding activated carbon to his 58-head herd in 2017 in an effort to reduce the impact from suspected silage contamination (Haines 2018). Affected animals were fed biochar which not only improved their health, it also helped boost production levels and a small increase in fat levels.

USA: Fairvue Farm, Connecticut

Main Focus: Pyrolysis of Dairy manure

The first of up to ten demonstrations of on-farm pyrolysis of dairy manure is located in Woodstock, CT at Fairvue Farms, a farm with 1,500 milking Holsteins that produce roughly 10 gallons of milk per cow per day. This collection of demonstrations is partially funded by USDA NRCS and uses Biomass Control's Biogenic Refinery (BR) to convert dairy manure into biochar. Native Energy, a carbon offset provider and project developer is identifying appropriate small farms for the project.



Much of the manure generated at Fairvue is land applied; however, there is an excess amount available. The current BR is able to process the manure from approximately 200 cows. Manure is collected from a storage pit below the milking barns and sent to either a separator shed or manure storage shed. A screw press reduces moisture from 90% to between 60 - 65%. Much of this dewatered manure is

Biomass Control unit in tractor trailer with feedstock augered in from bin outside of trailer.

used for bedding though there can, at times, be too much material for the farm's needs. When running full time, the BR can produce roughly 1 m^3 of biochar per day from 5 m^3 of dewatered manure.

USA: Shelburne Farms, Vermont

Main Focus: Odor control from manure slurry

In an effort to reduce odors emanating from manure storage facilities at Shelburne Farms, a non-profit dairy farm in Vermont focused on sustainable farming education, a truckload of biochar was applied to a 1,325 cubic meter manure slurry. A noticeable reduction in odor was observed once the biochar developed a cake on top of the slurry. While no peer-reviewed studies were produced from this work, farm management was pleased with a new option for odor management (Gribkoff 2019).

USA: Ontario Agricultural Commodities, California

Main Focus: Co-composting with dairy manure

Ontario Agricultural Commodities, a commercial-scale composter, teamed up with the Local Carbon Network in 2019 to pilot co-composting of dairy manure and biochar. The biochar was produced using the All Power Labs gasifier and is certified both by IBI and is listed by the Organic Materials Review Institute (OMRI) (All Power Labs 2019). Using a blend of 10% biochar and 90% dairy manure the piles not only reached consistently higher temperatures but were finished eight days sooner than the control pile with no biochar, representing a 30% reduction in finishing time. Hotter temperatures can help eliminate pathogens that may reside in the dairy manure.

DISCUSSION AND FUTURE RESEARCH

A growing number of dairy farmers have demonstrated interest in using thermo-chemical conversion of manure and the resulting biochar in different ways in their dairy operations. Often their interest stems from the need to find more cost-effective and environmentally benign manure management practices. Even though these pioneers are showing various ways to produce and use biochar on dairies, significantly more work is needed to demonstrate how and why the dairy industry should adopt these practices.

Quantification of GHG reductions using biochar on Dairy Farms

Pyrolysis has been recognized by the Intergovernmental Panel on Climate Change (IPCC) as one of only a handful of negative emission technologies (NETs). In addition, biochar used in soils has recently been added to the IPCC's list of mechanisms for countries to reach their Nationally Determined Contributions (NDC), or reduction commitments.

While the U.S. recently pulled out of the Paris Agreement and thus is not committing to reduction targets at the federal level, a growing number of States are committing to ambitious net-zero carbon targets. As an example, New York State has committed to reduce emissions by 40% by 2030 and become net zero by 2050. California's ambitions for net zero are targeted to occur even earlier (in 2045). Both States have large dairy industries and these targets can therefore not be met, unless dairy emissions are significantly reduced if not fully eliminated.

Calculating the GHG impact of using TCC and biochar in combination with current manure best-management practices and as a feed additive are critical for all U.S. states, as well as other countries, to assess the most cost-effective methods to achieving their goals. Benchmarking the emissions related to current practices against those that incorporate biochar through Life Cycle Assessments (LCAs), should be a top research priority.

Once LCAs are published, protocols can be developed for carbon markets that may help farmers finance a transition from current practices to lower emitting ones. States such as New York will start to de-emphasize carbon credits for renewable energy as 100% renewable is already part of the strategy for getting to net zero. This may also be the case with other emission reduction strategies. Carbon removal strategies may become much more valued in the near future. For this reason, understanding the carbon sequestration potential for manure biochar is also critical.

Optimizing TCC & Biochar in Manure Management

While the use of biochar in various manure management strategies has been researched and trialed at small scales, insufficient work has been done on the use of dairy manure biochar specifically for use in dairy manure composting, lagoon covers, or anaerobic digesters to understand how best to optimize these synergies on-farm. Understanding the optimal size pyrolysis unit on dairy farms that already have ADs but generate excess digestate and perhaps could benefit from increased CH_4 production, isneeded. Case studies that assess the capital and operating costs of co-locating different manure management processes with pyrolysis will enable farmers and other potential funders (e.g. carbon market brokers) to understand which combinations work on different sized dairies located in different parts of the U.S..

In addition, an assessment of different technologies available to carbonize manure would be helpful. This would include a review of the costs, capacities and co-products of different gasification and pyrolysis technologies that can handle manure as well as a closer look at the potential revenue streams and/or cost savings that farmers may achieve. Understanding any ancillary equipment (e.g. pre or post processing of feedstock and/or biochar) required as well as labor hours and skill sets is also necessary.

Research on the impact of feed biochar on milk production

While the benefits to animal health and to the environment from the addition of biochar to livestock feed is increasingly studied, few if any published papers exist on the long-term impact of feed biochar on milk production (the authors are aware of one on-going study in Australia on this topic as well as anecdotal discussions on dairies in the U.S. that implemented this approach with positive results but no published papers were found). It is critical to understand the impact on both volume and quality of milk production when dairy cattle routinely ingest biochar as a feed additive.

Until, or unless, extension agents, nutritionists, veterinarians and others are convinced of both the safety and benefits of adding biochar to daily feed, it may be challenging to scale its use on U.S. dairies beyond those dairies that are already pioneering these and similar efforts.

Demonstrations of biochar production & use on Dairy farms

Even though there are a growing number of dairies that are using and even producing biochar from manure or other organic sources, their numbers are still very small, and few people have access to these farms to learn from their experiences. Setting up on-farm pilot projects in different geographic locations, on farms of different sizes and manure management practices that others could visit would be helpful in demonstrating to other dairy farmers how the process works, what equipment and other assets are required and how the biochar can be used on-farm.

On-going coordination amongst dairy and biochar projects

In order for the lessons learned and best practices related to TCC and biochar use on dairies to be shared effectively within the industry, it is important to organize on-going coordination amongst dairy farmers that are piloting these practices. This would include outreach on a regular basis (e.g. semi-annual); scheduling virtual calls with other participating dairies; and documenting and sharing of issues, challenges, benefits, improvements and other feedback from dairies. In researching on-farm experiences for this white paper, most pioneering dairies venturing into the biochar space had little knowledge of other dairy farms involved with biochar. If there was some coordinating entity, new adopters of biochar-dairy approaches could potentially get up to speed quicker while avoiding challenges already overcome by others, thus further enabling scale.

CONCLUSIONS

As with most types of farming, the use of biochar and conversion of excess organics produced on farms is still at the earliest stages, though it is certainly beginning to garner more and more attention due to the multiple benefits offered. It is probably not over-stating the situation to say that a majority of those involved in the dairy industry, both in the production and processing have yet to have even hear about biochar. Still others that have heard about it may be skeptical as to the net benefit to the industry.

To date little, if any, targeted educational materials have been created and deployed to educate the dairy industry on the benefits and uses of biochar. This White Paper, a discussion with dairy specialists from Cornell Cooperative Extension as well as a webinar hosted by the International Biochar Initiative on this topic will help, but significantly more resources are required to educate extension agents, farmers, national, regional and state dairy associations, policy makers and others about the economic and environmental benefits which can be derived from pyrolyzing manure into biochar and using the manure biochar both on- and off-farm.

Attending and presenting at various industry gatherings, and more generally farming trade shows, professional conferences, and other events would help raise awareness and identify opportunities and hurdles to adoption. Writing articles and highlighting farmers that are already involved with TCC and biochar for different dairy publications, newsletters and journals would also be needed to reach as wide an audience as possible. Not only should producers be educated about these benefits, but buyers of milk products should also be more aware, particularly those that are focused on reducing their emissions throughout the supply chain. This includes both large buyers (e.g. fast food chains or ice cream, yogurt and cheese manufacturers) and small buyers that may be concerned about the carbon footprint of milk products.

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The use of biochar in animal feeding

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ABSTRACT

Biochar, that is, carbonized biomass similar to charcoal, has been used in acute medical treatment of animals for many centuries. Since 2010, livestock farmers increasingly use biochar as a regular feed supplement to improve animal health, increase nutrient intake efficiency and thus productivity. As biochar gets enriched with nitrogen-rich organic compounds during the digestion process, the excreted biochar-manure becomes a more valuable organic fertilizer causing lower nutrient losses and greenhouse gas emissions during storage and soil application. Scientists only recently started to investigate the mechanisms of biochar in the different stages of animal digestion and thus most published results on biochar feeding are based so far on empirical studies. This review summarizes the state of knowledge up to the year 2019 by evaluating 112 relevant scientific publications on the topic to derive initial insights, discuss potential mechanisms behind observations and identify important knowledge gaps and future research needs. The literature analysis shows that in most studies and for all investigated farm animal species, positive effects on different parameters such as toxin adsorption, digestion, blood values, feed efficiency, meat quality and/or greenhouse gas emissions could be found when biochar was added to feed. A considerable number of studies provided statistically non-significant results, though tendencies were mostly positive. Rare negative effects were identified in regard to the immobilization of liposoluble feed ingredients (e.g., vitamin E or Carotenoids) which may limit long-term biochar feeding. We found that most of the studies did not systematically investigate biochar properties (which may vastly differ) and dosage, which is a major drawback for generalizing results. Our review demonstrates that the use of biochar as a feed additive has the potential to improve animal health, feed efficiency and livestock housing climate, to reduce nutrient losses and greenhouse gas emissions, and to increase the soil organic matter content and thus soil fertility when eventually applied to soil. In combination with other good practices, co-feeding of biochar may thus have the potential to improve the sustainability of animal husbandry. However, more systematic multi-disciplinary research is definitely needed to arrive at generalizable recommendations.

Subjects Agricultural Science, Ecology, Soil Science, Veterinary Medicine, Environmental Impacts
Keywords Livestock emissions, Biochar feed, Mycotoxins, Animal health, Feed efficiency, Pesticides, Animal digestion, Enteric methane emissions, Redox activity

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INTRODUCTION

Biochar is produced by pyrolysis from various types of biomass in a low-to-no oxygen thermal process at temperatures ranging from 350 to 1,000 °C (*European Biochar Foundation (EBC), 2012*; *International Biochar Initiative (IBI), 2015*). Using water vapor or CO_2 at temperatures above 850 °C or chemical compounds like phosphoric acid and potassium chloride, the biochar undergoes an activation process resulting in activated biochar (i.e., activated carbon) (*Hagemann et al., 2018*). When produced from pure stem wood, the solid phase of the pyrogenic process is known as *charcoal*. In contrast, the term *biochar* indicates that a broad spectrum of biogenic materials can serve as feedstock. Biochar, activated carbon and charcoal can all be considered as pyrogenic carbon materials.

The term biochar indicates that it is used for any purpose that does not involve its rapid mineralization to CO_2 (e.g., burning it) (*European Biochar Foundation (EBC), 2012*). In a broader sense, the term *biochar* denotes its intended long-time residence in the terrestrial environment, either as a soil amendment or for other material-use purposes (*Schmidt et al., 2018*). Since biochar-carbon decomposes much slower than the original biomass, the application and use of biochar is considered as a terrestrial carbon sink on at least a centennial scale (*Zimmerman & Gao, 2013*; *Lehmann et al., 2015*; *Werner et al., 2018*) and is therefore a promising negative emission technology (*IPCC, 2018*).

During the first decade of modern biochar research summarized in *Lehmann & Joseph (2015)*, biochar was usually tested as a soil amendment that was applied pure to soils in large quantities (>10 t/ha) revealing modest to large yield increases for a multitude of crops in the tropics but only rarely in temperate climates (*Jeffery et al., 2017*). More recently it was (re-)discovered that blending biochar with organic amendments such as manure, cattle urine or compost may increase yields more significantly and in a broader spectrum of climates and soils (*Steiner et al., 2010*; *Kammann, Glaser & Schmidt, 2016*; *Godlewska et al., 2017*; *Schmidt et al., 2017*). As quality biochar is non-toxic and thus even feedable and edible (*European Biochar Foundation (EBC), 2012*), this apparently favorable combination of organic residues with biochar prompted researchers and a rapidly increasing number of practitioners to conduct trials where biochar was not only mixed with manure but also included as an input into animal farming systems. The incremental addition of biochar to silage, feed, bedding material and liquid manure pit demonstrated that biochar can be used in cascades. In addition to the direct benefits for animal husbandry as discussed below in detail, biochar becomes thus enhanced with organic nutrients which increases the economic viability of biochar application while providing numerous environmental benefits along the (cascading) way.

When combined with silage, biochar can reduce mycotoxin formation, bind pesticides, suppress butyric acid formation and enhance the quantity of lactic bacteria (*Calvelo Pereira et al., 2014*). Farmers observed that when biochar was combined with straw or saw dust bedding at 5–10% (vol) hoof diseases, odors and nutrient losses were reduced (*O'Toole et al., 2016*). Moreover, farmers reported that adding 0.1% biochar (m/m) in a liquid manure pit reduced odors, surface crust and nutrient losses (*Schmidt, 2014*; *Kammann et al., 2017*). Throughout these cascades, the biochar becomes enriched with organic nutrients and functional groups, while the cation exchange capacity and redox activity increases, and pH decreases (*Joseph et al., 2013*). Analyses indicate that, by enriching the biochar with liquids organic nutrients (whether in the digestive tract, bedding, manure pit or by co-composting), the interior surfaces of the porous biochar become drenched with an organic coating (*Hagemann et al., 2017*; *Joseph et al., 2018*). This increases both water storage capacity and nutrient exchange capacity (*Conte et al., 2013*; *Kammann et al., 2015*; *Schmidt et al., 2015*). The biochar becomes thus a more efficient plant growth enhancing soil amendment, that improves the recycling of nutrients from organic residues of animal farming (*Kammann et al., 2015*). The cascading use of biochar in animal farming systems also reduces the environmentally harmful loss of ammonia through volatilization or nitrate through leaching (*Liu et al., 2018*; *Borchard et al., 2019*; *Sha et al., 2019*) and it has the potential to reduce greenhouse gas emissions such as nitrous oxide (N_2O) (*Kammann et al., 2017*; *Borchard et al., 2019*), or methane (CH4) (*Jeffery et al., 2016*). To the best of our knowledge, no study so far has quantified biochar emission reduction effects along a full cascade. The studies cited above are reviews or meta-analyses summarizing mainly effects of the amendment of biochar to soil.

When in 2012 the cascading use of biochar and especially its addition to animal feed began in Germany and Switzerland (*Gerlach & Schmidt, 2012*), the biochar market in Europe started to grow considerably. Since then, the largest proportion of industrially produced biochar in Europe is sold for animal feed, bedding, manure treatment and thus subsequent soil application (*Kammann et al., 2017*; *O'Toole et al., 2016*; *Schmidt & Shackley, 2016*). In 2016, the European Biochar Foundation introduced a new biochar certification standard specifically for animal feed (*European Biochar Foundation (EBC), 2018*) to allow for quality control, as well as conformity with European regulations for animal feed.

When ingested orally, biochar has been shown to improve the nutrient intake efficacy, adsorb toxins and to generally improve animal health (*O'Toole et al., 2016*; *Toth & Dou, 2016*). After numerous veterinary papers published last century, a number of scientific studies on biochar feeding have been published since 2010, dealing with biochars' impact on the health of various animal species, on feed efficiency, pathogen infestation and on greenhouse gas emissions. Thus, we review the current state of knowledge regarding the use of biochar as a animal feed additive. We identify systematic gaps in the scientific understanding as it is still mechanistically unclear why biochar, as a feed additive, causes the observed effects. We also highlight potential side effects, the known and potential effects on greenhouse gas emissions, the necessity for adapted regulatory practice and quality control as well as the need for dedicated research to close knowledge gaps.

RESEARCH METHODS

This study predominantly selected research papers published between 1980 and 2019 but included also a selection of historical articles and books published between 1905 and 1979. Some rare oral communications were included to reference and illustrate farmer and feed certifier experiences.

Search strategy

We searched the following electronic databases: Science Direct, Scopus, ISI Web of Science and Research Gate. To identify the relevant publications, we used the following search terms: (biochar OR charcoal OR activated carbon) and (animal OR feed OR livestock OR livestock type (cow, poultry, sheep etc.) OR methane OR pesticides OR silage OR manure). The references cited in the reviewed studies were also included in the search and scanned separately for relevant publications. To summarize the historical literature (20 studies) we used the Karlsruhe Virtual Catalogue and the literature cited in the respective historical works in English, German and French. We further interviewed Dr. Achim Gerlach, a veterinarian who has been treating large cattle herds with biochar for nearly a decade; only a small fraction of his experiences are published in peer-reviewed journals (*Gerlach & Schmidt, 2012*).

Selection of studies

The authors assessed the titles and abstracts of all retrieved references of relevance to the objective of this review. Due to the relatively small number of studies, we included all studies that investigated biochar or charcoal or activated carbon in vivo as feed additive for improving performance and animal health (27 studies). We further selected in vivo or in vitro studies when animal tissue or digestive liquids were used as medium and if they were related to mycotoxin- (26 studies), bacteria related pathogen- (22 studies), poisoning and drug overdoses (21 studies), and pesticide- (23 studies) adsorption or methane emissions (12 studies). In total, 112 scientific studies on biochar effects in animal feeding were reviewed. Reported results were only discussed as significant when $p < 0.05$ was obtained in the respective study.

RESULTS AND DISCUSSION

Historical overview

The use of biochar/charcoal as feed or feed additive before 2010

Charcoal is one of the oldest remedies for digestive disorders, not only for humans but also for livestock. Cato the Elder (234 -149 BC) was one of the first to mention it in his classic *On Agriculture*: "If you have reason to fear sickness, give the oxen before they get sick the following remedy: 3 grains of salt, 3 laurel leaves, [. . .], 3 pieces of charcoal, and 3 pints of wine." (*Cato, 1935*, §70). Besides the administration of medicinal herbs, oil or clay, charcoal was widely used by traditional farmers all over the world for internal disorders of any sort. Apparently, it never did any harm but was mostly beneficial (*Derlet & Albertson, 1986*). For some animals like chicken or pigs, the charcoal was administered pure; for others it was mixed with butter (cows), with eggs (dogs) or with meat (cats).

A textbook on animal husbandry dating from 1906 observed: "Swine appear to have a craving for what might be called 'unnatural substances'. This is especially true of hogs that are kept in confinement, which will eat greedily such substances as charcoal, ashes, mortar, soft coal, rotten wood etc. It is probable that some of the substances are not good for hogs, but there is no doubt that charcoal and wood ashes have a beneficial effect, the former being greatly relished" (*Day, 1906*).

19th century and early 20th century agricultural journals printed many discussions on the benefits of various "cow tonics," mostly composed of charcoal and a variety of other ingredients including spices, such as cayenne pepper, and digestive bitters like gentian. Manufacturers of these tonics claimed they would reduce digestive disorders, increase appetite and improve milk production (*Pennsylvania State College, 1905*).

At this time in the USA, charcoal was considered a superior feed additive for increasing butterfat content of milk. Cow's milk was tested for butterfat content in competitions where top-producing cows could win a prize. Farmers took great care in formulating the feed ration for such tests: *The grain mixture fed during the test consisted of 100 pound of distillers dried grains, 50 pounds of wheat bran, 100 pounds of ground oats, 100 pounds of hominy, 100 pounds of cottonseed meal . . . . Charcoal is seldom if ever left out the test ration by many of the breeders*" (*Savage, 1917*).

The use of activated and non-activated biochar feed for animal health was already being researched and recommended by German veterinarians at the beginning of the last century. Since 1915, research into activated biochar had revealed its effect in reducing and adsorbing pathogenic clostridial toxins from *Clostridium tetani* and *Clostridium botulinum* (*Skutetzky & Starkenstein, 1914*; *Luder, 1947*). *Mangold (1936)* presented a comprehensive study on the effects of biochar in feeding animals, concluding that *"the prophylactic and therapeutic effect of charcoal against diarrheal symptoms attributable to infections or to the type of feeding is known. In this sense, adding charcoal to the feed of young animals would seem a good preventive measure."* *Volkmann (1935)* described an effective reduction in excreted oocysts through adding biochar to the food of pets with coccidiosis or coccidial infections.

Later, *Totusek & Beeson (1953)* wrote that biochar products are used since at least 1880 in US-American hog breading and since 1940 in feed for poultry. In their influential article, the authors provided an extensive list of references. At around the same time, *Steinegger & Menzi (1955)* wrote: "*It is generally common in Switzerland to add biochar to chick feed and to the meal for laying hens to prevent digestive problems and to achieve a regulating effect on digestion.*"

Biochar and wild animals

At first glance it might seem somewhat unnatural to feed biochar/charcoal to animals, but in fact even wild mammals occasionally eat biochar if it is available to them. In nature, charcoal residues from wild fires can still be found years later. Deer and elk are reported to eat from charred trees in Yellowstone National Park and domestic dogs to eat charcoal briquettes (*Struhsaker, Cooney & Siex, 1997*). The *Zanzibar red colobus* (*Procolobus kirkii*), a small monkey regularly eats charcoal to help digest young Indian Almond (*Terminalia catappa*) or mango (*Mangifera indica*) leaves that contain toxic phenolic compounds (*Cooney & Struhsaker, 1997*). *Struhsaker, Cooney & Siex (1997)* observed that individual colobus monkeys consumed about 0.25–2.5 g of charcoal per kg body weight daily. Additional adsorption tests performed by *Cooney & Struhsaker (1997)* indicated that in particular the African kiln charcoals (which the monkeys also ate) were surprisingly good at adsorbing hot-water-extracted organics from the above-mentioned tree leaves. Thus, the authors concluded that the monkeys' charcoal consumption was likely a (self-)learned behavior, increasing the digestibility of their typical leaf diet. Interestingly, a population count of *colobus* monkeys on this African island showed that they reached the highest population density of all monkey species worldwide. It seems, therefore, that the daily consumption of such wood-based biochar has no negative long-term effect at least not on these monkeys.

Mechanisms of biochar in feed digestion

Adsorption

Before biochar was investigated and used as a regular feed additive for animals in the early 2010s, charcoal (i.e., biochar made from wood) and activated carbon (i.e., activated biochar when made from biomass; *Hagemann et al., 2018*) was considered a veterinary drug to tackle indigestion and poisoning. Charcoal was known for many centuries as an emergency treatment for poisoning in animals (*Decker & Corby, 1971*). Biochar has been and still is used because of its high adsorption capacity for a variety of different toxins like mycotoxins, plant toxins, pesticides as well as toxic metabolites or pathogens. Adsorption therapy, which uses activated biochar as a non-digestible sorbent, is considered one of the most important ways of preventing harmful or fatal effects of orally ingested toxins (*McKenzie, 1991*; *McLennan & Amos, 1989*).

From a toxicology perspective, most of the effects of biochar are based on one or several of the following mechanisms: selective adsorption of some toxins like dioxins, co-adsorption of toxin containing feed substances, adsorption followed by a chemical reaction that destroys the toxin and desorption of earlier adsorbed substances in later stages of digestion (*Gerlach & Schmidt, 2012*). However, classifiable distinctions need to be made to the time-dependent and partly overlapping processes of adsorption, biotransformation, desorption and excretion of the toxic substances throughout the digestive system of animals.

Schirrmann (1984) described the effects of activated carbon on bacteria and their toxins in the gastrointestinal tract as:

1. Adsorption of proteins, amines and amino-acids.
2. Adsorption of digestive tract enzymes, as well as adsorption of bacterial exoenzymes.
3. Binding, via chemotaxis, of mobile germs.
4. The selective colonization of biochar with gram-negative bacteria might result in decreased endotoxin release as these toxins could be directly adsorbed by the colonized biochar when gram-negative bacteria dying-off.

One further major advantage of the use of biochar is its "enteral dialysis" property, that is, already adsorbed lipophilic and hydrophilic toxins can be removed from the blood plasma by the biochar, as the adsorption power of the huge surface area of the biochar interacts with the permeability properties of the intestine (*Schirrmann, 1984*).

Susan *Pond (1986)* explained various mechanisms by which biochar can eliminate toxins from the body. First, biochar can interrupt the so-called enterohepatic circulation of toxic substances between the intestine, liver and bile. It prevents compounds such as estrogens and progestagens, digitoxin, organic mercury, arsenic compounds and indomethacin from being taken up in bile. Second, compounds such as digoxin, which are actively secreted into the intestine, can be adsorbed there. Third, compounds such as pethidines can be adsorbed to the biochar, which passively diffuse into the intestine. Fourth, the biochar can take up compounds that diffuse along a concentration gradient between intestinal blood and primary urine.

Redox activity of biochar-based feed additives

Although the adsorption capacity is the most prominent function of biochar to explain its positive impacts when fed to animals, adsorption alone cannot explain all phenomena that are observed in biochar feeding experiments. Another pivotal, but still widely overlooked function of biochar is its redox activity. Biochars act as so called *geobatteries and geoconductors* that can accept, store and mediate electrons from and for biochemical reactions (*Sun et al., 2017*). Low temperature biochars (HTT of 400–450 °C) function as geobatteries mainly due to their phenol and quinone surface groups. High temperature biochars (HTT >600°), on the other hand, are good electrical conductors (*Mochidzuki et al., 2003*; *Yu et al., 2015*). Due to both of these qualities, both, high and low temperature biochars, can act in biotic and abiotic redox-reactions as electron mediators (*Van Der Zee & Cervantes, 2009*; *Husson, 2012*; *Liu et al., 2012*; *Kappler et al., 2014*; *Kluepfel et al., 2014*; *Joseph et al., 2015a*; *Yu et al., 2015*; *Sun et al., 2017*). Biochar can accept and donate electrons as, for example, in microbial fuel cells where activated biochar can be used as an anode and as a cathode (*Gregory, Bond & Lovley, 2004*; *Nevin et al., 2010*; *Konsolakis et al., 2015*). The electrical conductivity of biochar is, however, not based on continuous electron flow, like in a copper wire, but on discontinuous electron hopping (*Kastening et al., 1997*), which is of essential importance for biochar's function as a (microbial) electron mediator or so-called electron shuttle, facilitating even inter-species electron transfer (*Chen et al., 2015*). Due to the comparably large size of biochar particles, the electron transfer capacity of biochar's carbon matrices may lead to a relatively long-distance electron exchange that provides a spatially more extensive accessibility to alternative electron acceptors such as minerals for anoxic microbial respiration (*Sun et al., 2017*).

During the microbial decomposition of organic substances in the gastrointestinal tract and particularly in the anaerobic rumen, digestive microbes require a terminal electron acceptor to get rid of surplus electrons that accumulate during the degradation of organic molecules. As electrons do not exist in a free state under ambient environmental conditions and cannot be stored in large enough quantities by cells, organisms always depend on the availability of both an electron donor (e.g., the metabolized organic matter) and an acceptor to which surcharge electrons can be transferred. This usually occurs in so-called redox reactions where molecules or atoms that donate an electron are coupled through electro-chemical reactions with molecules or atoms that accept an electron. To allow this electron transfer, these chemical or biochemical redox-reactions usually have to take place in very close (molecular) proximity.

The coupling of electron donating and electron accepting reactions can, however, be bridged by so-called electron mediators or electron shuttles. Those electron meditators can take up an electron from a chemical reacting molecule, solid interphase or microorganism and provide it to another molecule, atom, solid interphase or microorganism. Well known and investigated electron mediating compounds include thionine, tannins, methyl blue or quinone, showing comparable capacities to humic substances and biochar (*Van Der Zee et al., 2003*; *Liu et al., 2012*; *Bhatta et al., 2012*; *Kluepfel et al., 2014*).

A well-balanced animal feed regime should contain multiple electron mediating substances. In the high-energetic diets used in intensive livestock farming, the supply with electron-shuttling substances is, however, often insufficient (*Sophal et al., 2013*). When inert or other non-toxic electron mediators like biochar or humic substances are added to high-energy feed, several redox reactions may take place more efficiently, which could in turn increase the feed intake efficiency (*Liu et al., 2012*; *Leng, Inthapanya & Preston, 2013*). Biochar, specifically, can act as both a sole electron mediator or a synergistic electron mediator that increases the efficiency of other mediators (*Kappler et al., 2014*).

Inside the gastro-intestinal tract, nearly all feed-degrading reactions are facilitated by microorganisms (mostly bacteria, archaea and ciliates). Within those reactions, bacterial cells may transfer electrons to biofilms or via biofilms to other terminal electron acceptors (*Richter et al., 2009*; *Kracke, Vassilev & Krömer, 2015*). However, biofilms are rather poor electric conductors and the electron-accepting capacity is low. Hence, microbial redox reactions can be optimized by electron shuttles, such as humic acids or activated biochar whose electrical conductivity is 100–1,000 times higher than that of biofilms (*Aeschbacher et al., 2011*; *Liu et al., 2012*; *Saquing, Yu & Chiu, 2016*). Although the conductivity of non-activated biochar is lower compared to activated biochar, it has been shown that it can efficiently transfer electrons between bacterial cells (*Chen et al., 2015*; *Sun et al., 2017*). Bacteria were shown to donate an electron to a biochar particle while other bacteria of different species took up (accepted) an electron at another site of the same biochar particle. The biochar acts here like a "battery" (or electron buffer) that can be charged and discharged, depending on the need of biochemical (microbial) reactions (*Liu et al., 2012*). Moreover, as biochar can be temporarily oxidized or reduced by microbes (i.e., biochar is depleted or enriched in electrons), it can buffer situations with a (temporary) lack of electron donors or terminal electron acceptors (redox buffering effect) (*Saquing, Yu & Chiu, 2016*). A principal aim of feeding biochar to animals could thus be to overcome metabolic redox limitations by enhancing electron exchange between microbes, and between microbes and terminal electron acceptors.

The redox-active carbonaceous backbone of the biochar as well as minerals it contains, such as iron (Fe(II) and/or Fe(III)) and manganese (Mn(III) or Mn(IV) minerals), can electrically support microbial growth in at least four different ways: (1) as an electron sink for heterotrophy-based respiration, (2) as an electron sources for autotrophic growth, (3) by enabling cell-to-cell transfer of electrons and (4) as an electron storage material (*Shi et al., 2016*). It can be hypothesized that enabling of extracellular electron transfer contributes to a more energy efficient digestion resulting in higher feed efficiency when activated or non-activated biochar is administered. Moreover, the electrochemical effects need to be considered as a major factor for explaining possible shifts in the functional diversity of the microbial community in the digestive system (*Prasai et al., 2016*). *Leng, Inthapanya & Preston (2012)* also suggested that electron transfer between biochar and microorganisms could be one of the reasons why feeding biochar to cows led to reduced methane emissions in their studies (see chapter 6).

It is further very likely that biochar has the function of a redox wheel in the digestive tract, comparable to Fe^{III}–Fe^{II}-redox wheels. It could act jointly as an electron acceptor and donator coupling directly various biotic and abiotic redox-reactions comparable to mixed valent iron minerals (*Davidson, Chorover & Dail, 2003*; *Li et al., 2012*; *Joseph et al., 2015a*; *Quin et al., 2015*). Beside its polyaromatic backbone, biochar contain, depending on the production process, a multitude of volatile organic carbons (VOC) (*Spokas et al., 2011*). Some of the pyrolytic VOCs are strong electron acceptors and may act, like a redox wheel similar to how quinone works (*Van Der Zee et al., 2003*). Some of these pyrolytic VOCs that often undergo oxidative modifications during the aging of biochar (*Cheng & Lehmann, 2009*) are so-called redox-active moieties (RAMs) that have been shown to contribute to the biodegradation of certain contaminants (*Yu et al., 2015*). It can be surmised that in the digestive tract, a multitude of RAMs, adsorbed on the surfaces of biochar particles, can act as redox-wheels with various microorganisms. It can be further hypothesized that when biochar buffers electrons in the vicinity of redox active surface groups, it may provide stabile micro-habitats with different redox-pH-milieus for different species of microorganisms (*Yu et al., 2015*). Moreover, biochar adsorbs certain feed and metabolic substances like tannins, phenols or thionin, which are also electron acceptors and which might further increase the electron buffering of biochar particles during its passage through the digestive tract (*Kracke, Vassilev & Krömer, 2015*).

Biochar, wood vinegar (i.e., aqueous solutions of condensed pyrolytic gases) and humic substances can act as redox buffering substances (*Husson, 2012*; *Kluepfel et al., 2014*) which may explain why the feeding of biochar, pyrolytic vinegar and humic substances often show similar effects; and why the blending of biochar with wood vinegar or humic substances seems to reinforce the effects (*Watarai, Tana & Koiwa, 2008*; *Gerlach et al., 2014*). However, unlike both dissolved organic substances, biochar provides a highly porous framework with high specific surface area, where humic-like substances or pyrolytic vinegar can be adsorbed and unfurl three-dimensionally as a coating of the inner-porous aromatic carbon surfaces of biochar. Due to the redox buffering effect of biochar blended with humic substances or wood vinegar, variations of the redox potential may be minimized in the proximity of biochar particles, which could support those species of microorganisms that find their optimum at these redox potentials (*Kalachniuk et al., 1978*; *Cord-Ruwisch, Seitz & Conrad, 1988*). Biochar particles may thus provide selective hotspots of microbial activity. It can be assumed that the buffering of the redox potential as well as the effect of electron shuttling between microbial species can have a selective, microbial milieu forming effect, which facilitates and accelerates the formation of functional microbial consortia (*Kalachniuk et al., 1978*; *Khodadad et al., 2011*; *Sun et al., 2017*).

The mechanistic understanding of biochar used as feed additive, especially with regard to its impact on microbial mediated redox reactions, is clearly in its infancy (*Gregory, Bond & Lovley, 2004*; *Nevin et al., 2010*; *Konsolakis et al., 2015*). However, we hypothesize with some confidence that biochar has a direct electro-chemical influence on digestive reactions, and that this is one, if not the main, reason for the extremely varying effects of different biochars. Electrical conductivity, redox potential, electron buffering (poising) and electron transfer capacity (shuttling) of a given biochar depend highly on the type of pyrolyzed feedstock, pyrolytic conditions (*Kluepfel et al., 2014*; *Yu et al., 2015*) and especially on pyrolysis temperature (*Sun et al., 2017*). The higher the temperature above 600 °C, the better is the electron transfer rate and electrical conductivity (*Sun et al., 2017*). However, the higher the VOC content of, for example, lower-temperature biochars and higher abundance of surface functional groups on lower temperature biochars (400–600 °C), the more important the mediated electron transfer onto/from the biochar may become (*Joseph et al., 2015a*; *Yu et al., 2015*; *Sun et al., 2017*). In addition, the mineral content of biochars should be taken into account as well, since it does not only influence biochar's electro-chemical behavior, but it may also catalyze various biotic and abiotic reactions (*Kastner et al., 2012*; *Anca-Couce et al., 2014*).

Specific toxin adsorption

Adsorption of mycotoxins

The contamination of animal feed with mycotoxins is a worldwide problem that affects up to 25% of the world's feed production (*Mézes, Balogh & Tóth, 2010*). Mycotoxins are mainly derived from mold fungi, whose growth on fresh and stored animal feed is difficult to prevent, especially in humid climates. Mycotoxin-contaminated feed can result in serious diseases of farm animals. To protect the animals, adsorbents are usually added to the feed to bind the mycotoxins before ingestion. In addition to the frequently used aluminosilicates, activated carbon and special polymers are increasingly being used (*Huwig et al., 2001*).

One of the most common mycotoxins is aflatoxin (*Alshannaq & Yu, 2017*), which has, therefore, been used in numerous studies as a model substance to investigate the adsorption behavior of biochar and how it reduces the uptake of the toxin in the digestive tract and hence in the animal blood and in milk (*Galvano et al., 1996a*). *Galvano et al. (1996b)* were able to reduce the extractable aflatoxin concentration in animal feed by up to 74% and the concentration in milk by up to 45%, by adding 2% activated biochar to pelleted aflatoxin-spiked feed for dairy cows. The non-systematic comparison of different activated biochars, however, showed that there are large differences in the adsorption efficiency between different types of (activated) biochar.

Diaz et al. (2002) showed in an in vitro sorption batch study that four different activated carbons adsorbed 99% of the aflatoxin B from a 0.5% aflatoxin B-spiked solution when activated biochars were dosed at 1.11 g on 100 ml. However, when Diaz administered 0.25% activated carbon to aflatoxin-B contaminated feed for dairy cows a year later (*Diaz et al., 2004*), they were unable to demonstrate any significant reduction in aflatoxin B levels in the milk. Here, it has to be considered that in the in vivo test, an insufficiently characterized (activated) biochar was fed at a low concentration of 0.25% of the feed fresh weight, whereas in the in vitro studies, the biochar was added at 1% to the aqueous solution, that is, four times higher, and in the absence of a feed matrix.

Galvano et al. (1996a) also investigated the adsorption capacity of 19 different activated carbons for two mycotoxins, ochratoxin A and deoxynivalenol, and found that the activated biochar adsorbed 0.80–99.86% of the ochratoxin A and up to 98.93% of the deoxynivalenol, depending on the type of activated biochar. The large range of results clearly confirms the importance of a systematic characterization and classification of biochar properties. However, Galvano et al. concluded that neither the iodine number used for activated biochar characterization, nor the Brunauer–Emmet–Teller specific surface area derived from N_2 gas-adsorption isotherms allowed straightforward predictions of the adsorption capacity for these mycotoxins.

Di Natale, Gallo & Nigro (2009) compared various natural and synthetic adsorbent feed additives for dairy cows to reduce the aflatoxin content in milk. Activated biochar showed the highest toxin reduction capacity (>90% aflatoxin reduction in milk with 0.5 g aflatoxin per kg diet). Analytical studies of the milk quality also showed slight positive effects on the milk composition with regard to organic acids, lactose, chlorides, protein content and pH. The authors explained the high adsorption capacity with the high specific surface area in combination with a favorable micropore size distribution of the biochar, and the high affinity of aflatoxin for the polyaromatic surface of the biochar in general (*Di Natale, Gallo & Nigro, 2009*).

Bueno et al. (2005) investigated the adsorption capacity of various doses of activated biochar (0.1%, 0.25%, 0.5%, 1%) for zearalenone, a dangerous estrogenic metabolite of the fungus species Fusarium, for which so far no treatment agents had been found. In vitro, all zearalenone could be bound at each of the four biochar doses. However, in vivo, where a wide variety of mycotoxins and numerous other organic molecules compete with the free adsorption surfaces of biochar, hardly any specific adsorption could be achieved.

A study with Holstein dairy cows investigated to what extent the negative effects of fungal-contaminated feed silage can be reduced by co-feeding activated biochar at 0, 20 or 40 g daily (*Erickson, Whitehouse & Dunn, 2011*). Cows fed the biochar amendment and the contaminated silage had higher feed intake and improved digestibility of neutral detergent fiber, hemicellulose and crude protein and had higher milk fat content compared to the control without biochar. When the same daily amounts of biochar were administered to uncontaminated quality silage, no changes in digestion behavior, milk quality or any other effect on the dairy cows could be detected. However, the authors showed in a second experiment that cows, when given the choice, clearly preferred good quality silage to contaminated silage either with or without biochar. They concluded that farmers should focus on providing high quality feed rather than mitigating negative effects of contaminated silage with biochar.

While *Piva et al. (2005)* found no protection against the injurious effects of fumonisin, a highly toxic mycotoxin, following a 1% addition of biochar to the feed of piglets, Nageswara *Rao & Chopra (2001)* showed that the addition of biochar to aflatoxin B1 contaminated feed of goats reduced the transfer of the toxin (100 ppb) to the milk by 76%.

In the latter trial, the efficiency of activated biochar was significantly higher than that of bentonite (65.2%). Both adsorbents did not affect the composition of goat's milk nor the average level of milk production.

In vitro studies with porcine digestive fluids showed high rates of adsorption of *Fusarium* toxins such as deoxynivalenol (67%), zeralenone (100%) and nivalenol (21%) through activated biochar (*Avantaggiato, Solfrizzo & Visconti, 2005*; *Döll et al., 2007*). On the other hand, *Jarczyk, Bancewicz & Jedryczko (2008)* found no significant effect when they added 0.3% activated biochar to the diet of pigs. Neither in the blood serum nor in the kidneys, the liver or in the muscle tissue could the ochratoxin concentrations be reduced by this small amount of supplement with uncharacterized industrial biochar (*Jarczyk, Bancewicz & Jedryczko, 2008*). However, no adverse effect was noted either.

Mycotoxins often cause serious liver damage in poultry. Biochar administered at daily rates of 0.02% of the body weight significantly increased the activity of key liver enzymes (*Ademoyero & Dalvi, 1983*; *Dalvi & Ademoyero, 1984*). While aflatoxin (10 ppm) reduced feed intake and weight gain of broiler chickens, the addition of 0.1% biochar to the feed (w/w) reversed the negative trend (*Dalvi & McGowan, 1984*).

Comparing the effect of activated biochar with a conventionally used alumina product (hydrated sodium calcium aluminosilicate), it was found that the alumina product resulted in considerable liver and blood levels of aflatoxin B when administered at 0, 40, 80 μg AFB1 per kg diet, but not when combined with a 0.25% and 0.5% biochar treatment (*Kubena et al., 1990*; *Denli & Okan, 2007*). In another study, activated biochar reduced the concentration of aflatoxin B in the feces of chickens for fattening, but only if the biochar was administered separately from the feed (*Edrington et al., 1996*). However, *Kim et al. (2017)* showed with an in vivo pig feeding trial that the aflatoxin absorption capacity was reduced by 100%, 10% and 20%, respectively, for three different biochars supplemented at 0.5% to the same basal diet, again demonstrating the importance of considering specific biochar properties. The importance of dosage was confirmed in another recent poultry trial where 0.25% or 0.5% activated biochar was added to an aflatoxin B1 contaminated diet, decreasing aflatoxin B1 residues in the liver of the birds by 16–72%, depending on the aflatoxin B1 and biochar dosages (*Bhatti et al., 2018*).

In their review article, *Toth & Dou (2016)* document further conflicting studies in which biochar feeding may or may not mitigate the effects of mycotoxin intoxication. The results of most studies on sorption in aqueous solution (in vitro) did not correlate with the results in corresponding in vivo test results (*Huwig et al., 2001*). Thus, in vitro studies have to be interpreted with care, because matrix effects can dramatically impact mycotoxin sorption, for example, *Jaynes, Zartman & Hudnall (2007)* found that an activated carbon (Norit®, Boston, MA, USA) could sorb up to 200 g/kg aflatoxin, but only in clear solution. In a corn meal suspension, sorption capacity was 100 times lower due to matrix effects. Matrix effects in the digestive tract can be expected to be even more complex due to varying pH and redox conditions. Still, based on our review, we conclude that negative effects of certain mycotoxins such as deoxynivalenol (*Devreese et al., 2012*, *2014*; *Usman et al., 2015*) and zearalenone (*Avantaggiato, Havenaar & Visconti, 2004*) can be effectively suppressed with rather low dosages of activated biochar amended to feed, while no benefit was found for aflatoxin. It can be hypothesized that (activated) biochar is only able to suppress negative effects of mycotoxins that are rather hydrophobic (*Avantaggiato, Havenaar & Visconti, 2004*).

However, most of these studies have in common that only commercial activated carbons and biochars were used without proper characterization, that is, systematic trials with biochar of different feedstock (e.g., wood vs. herbaceous feedstock) and production conditions (e.g., temperature) are barely available. Thus, systematization of the results remains difficult.

Adsorption of bacteriological pathogens and their metabolites

The use of activated and non-activated charcoals to improve animal health was recommended and studied by German veterinarians as far back as the beginning of the 20th century. In 1914, the adsorbing effect of charcoal for various toxins in the digestive tract was described by *Skutetzky & Starkenstein (1914)*. First experiments with bacterial toxins of *Clostridium tetani* and *Clostridium botulinum* as well as with diphtheria toxin were performed as early as 1919 (*Jacoby, 1919*). In particular, Wiechowski pointed out how important the quality of the charcoal is, and how different the effect of different charcoals on the toxin adsorption can be (*Wiechowski, 1914*). Ernst Mangold described in 1936 the effect of charcoal in animal feeding comprehensively and concluded: "*The prophylactic and therapeutic effect of charcoal on infectious or feeding-related diarrhea is clear, and based on this observation, the co-feeding of charcoal to juvenile animals appears as an appropriate prevention*" (*Mangold, 1936*). At about the same time, Albert Volkmann published his findings about efficient reduction of oocyst excretion resulting from coccidiosis and coccidial infections when charcoal was fed to domestic animals (*Volkmann, 1935*).

Gerlach et al. (2014) demonstrated that daily supplement of 400 g of a high-temperature wood-based biochar (i.e., HTT 700 °C) significantly reduced the concentration of antibodies against the Botox-producing pathogen *Clostridium botulinum* in the blood of cattle indicating the suppression of the pathogen. They concluded that the neurotoxin concentration was reduced by the biochar in the gastrointestinal tract of the animals. The feeding of only 200 g of biochar per day did not show the same efficiency. However, when this lower dosage was mixed with 500 ml of lactobacilli-rich sauerkraut juice, a similar significant reduction of *Clostridium botulinum* antibodies in the blood could be measured.

Knutson et al. (2006) fed sheep infected with *Escherichia coli* and *Salmonella typhimurium* 77 g of activated biochar per animal per day. Although *Naka et al. (2001)* had shown earlier by in vitro trials that *E. coli* O157: H7 (EHEC) cell counts were reduced from 5.33×10^6 by five mg/ml activated biochar to below 800, the in vivo test by Knutson et al. with the same activated biochar (DARCO-KB; Norit®) revealed no biochar-related binding of either *E. coli* or *S. typhimurium* in the gastrointestinal tract of sheep. The authors hypothesized that either the biochar binding sites were occupied by competing substances or other digestive bacteria or that the time between infection with the pathogen and administration of the biochar was too long.

Schirrmann (1984) indicated that biochar has a particularly strong adsorption or suppression capacity for gram-negative bacteria (e.g., *E. coli*) with high metabolic activity (see more below in section "Administration of Biochar Feed and Biochar Quality Control": Side effects of biochar). Fecal *E. coli* counts in manure after feeding 0.25% activated biochar or 0.50% coconut tree biochar were significantly lower than those of the control without biochar in 10 days finishing pig trial, while the number of beneficial bacteria *Lactobacillus* in feces increased in both biochar treatments (*Kim et al., 2017*).

Liquid cattle manure often contains *E. coli* O157: H7 (EHEC), which can contaminate water and soil and enter the human food chain (*Diez-Gonzalez et al., 1998*). Biochar can both adsorb *E. coli* and its toxic metabolites already in the digestive tract, as well as reduce the spread of those bacteria in water and soil by adding it to manure. *Gurtler et al. (2014)* investigated the effect of various biochar on the inactivation of *E. coli* O157: H7 (EHEC) when applied to soils. All biochars produced by either fast or slow pyrolysis from switchgrass, horse manure or hardwood significantly reduced EHEC concentrations, with fast pyrolysis of barley and oak log feedstock providing the best results in the contaminated soil mix, where EHEC after 4 weeks were untraceable using a cultivation based assessment (*Gurtler et al., 2014*).

Abit et al. (2012) investigated how *E. coli* O157: H7 and *Salmonella enterica* spread in water-saturated soil columns of fine sand or sandy loam, when the soil columns were blended with 2% of different biochars. While chicken manure biochar prepared at 350 °C did not improve the binding of either bacteria, the addition of biochar prepared at 700 °C from pinewood or from chicken manure significantly reduced the spread of both bacteria. In a later study, the authors showed significant differences in immobilization between the two bacterial strains and suggested that the surface properties of the bacteria played a significant role in the binding of these bacteria to the biochar (*Abit et al., 2014*). The latter may turn out to be an important insight into biochar—bacterial interaction and needs to be investigated systematically.

Since *E. coli* infections are likely to spread through cattle herds via water troughs, the prophylactic addition of biochar to trough water may be a preventive measure that should be further investigated.

In the study of *Watarai & Tana (2005)*, the mixture of fodder with 1% and 1.5% bamboo biochar and bamboo vinegar, respectively, slightly but significantly reduced the levels of *E. coli* and *Salmonella* in chicken excrement. A patented biochar—wood vinegar product, *Nekka-Rich* (*Besnier, 2014*), whose composition was not revealed, showed a highly significant reduction of *Salmonella* in chicken droppings. It was further found that the biochar—wood vinegar product reduced the pathogenic gram-negative *Salmonella enterica* bacteria in the droppings, but not the intestinal flora of ubiquitous, non-toxic, gram-positive *Enterococcus faecium* bacteria (*Watarai & Tana, 2005*).

A 0.3% bamboo biochar feed supplement (on DM base) suppressed the fecal excretion of gram-negative coliform bacteria and gram-negative *Salmonella* in pigs up to 20- and 1,100-fold, respectively, compared to controls without biochar (*Choi et al., 2009*). The effect of biochar on the suppression of both bacterial species was of the same order of magnitude as that of antibiotics. Feeding biochar resulted in a 190-fold increase in the number of beneficial intestinal bacteria and a 48-fold higher level of gram-positive *Lactobacilli* compared to the treatment with antibiotics (*Choi et al., 2009*).

In vitro studies revealed that biochar, as well as clay, can efficiently immobilize cattle rotavirus and coronaviruses at rates of 79–99.99% (*Clark et al., 1998*). Since the diameter of the viral particles were larger than the pore diameters of the clay and most pores of the biochar, the authors suspected that binding was mainly due to the viral surface proteins binding to the biochar.

In vitro and in vivo experiments with bovine calves showed that biochar, especially in combination with wood vinegar, was able to control parasitic protozoan *Cryptosporidium parvum* infection and to stop diarrhea of calves within one day. The number of oocysts in the feces dropped significantly after a single day of feeding biochar; after 5 days no more oocysts could be found in the feces of the calves (*Watarai, Tana & Koiwa, 2008*). Similar results were reported when a commercial biochar wood acetic acid product (Obionekk®, Obione, Charentay, France) was tested as feed additive in young goats (*Paraud et al., 2011*). The mixture administered twice or thrice daily reduced the clinical signs of diarrhea already on the first day, and the oocyst shedding in the feces decreased significantly. Over the period of the study, the mortality of the young goats was 20% in the control group and only 6.7% in the treatment group that received Obionekk® three times per day. Biochar feeding in goats may also reduce the incidence of parasites such as cestode tapeworms and *coccidia* oocysts (*Van, Mui & Ledin, 2006*).

Adsorption of drugs

Numerous human medical studies on the use of activated carbon in poisoning have been published in the 1980s providing important insights into the use of (activated) biochar as feed especially to treat feed poisoning (*Erb, Gairin & Leroux, 1989*). The adsorbing effect of activated carbon can be used to prevent the gastrointestinal uptake of most drugs and numerous toxins (*Neuvonen & Olkkola, 1988*), which is typically more effective than pumping out stomach contents. The repeated intake of activated carbon or biochar improved the elimination of overdosed toxicologically effective substances such as aspirin, carbamazepine, dapsone, dextropropoxyphene, cardiac glycosides and many more as summarized by *Neuvonen & Olkkola (1988)*. Moreover, a faster elimination of many industrial and environmental toxins was assessed. In acute poisoning, 50–100 g of activated biochar are administered to adults and about one g/kg of body weight to children. The same authors also point out that there are no known serious side effects from accidental ingestion. In the case of acute poisoning, Finnish physicians recommend repeated oral treatment with activated carbon to reduce the risk of toxins being desorbed from the biochar-toxin complex in the digestive cycle (*Olkkola & Neuvonen, 1989*). In general, repeated oral administration of biochar increases the efficacy of detoxication (*Crome et al., 1977*; *Dawling, Crome & Braithwaite, 1978*). However, regular administration of 0.2% activated biochar in broiler feed did not significantly impact the blood levels of the antimicrobial drugs doxycycline and tylosin, and of the coccidiostats diclazuril and salinomycin. The pharmaceutical products were co-applied to the activated carbon amended feed (*De Mil et al., 2017*).

Adsorption of pesticides and environmental toxins

Based on the excellent adsorption properties of biochar in relation to numerous pesticides, insecticides and herbicides (*Safaei Khorram et al., 2016*; *Mandal, Singh & Purakayastha, 2017*; *Cederlund, Börjesson & Stenström, 2017*), which are increasingly found in animal feed (*Shehata et al., 2012*), biochar is considered as animal feed additive. Of particular importance is the adsorption of glyphosate, an herbicide that currently contaminates most of the feed produced from genetically modified maize, rapeseed and soybean. Although crop desiccation herbicides have been banned in Germany since May 2014, they are still permitted in many other countries as a treatment shortly before grain harvest. In addition to immobilizing magnesium and zinc, glyphosate has a potent antibiotic activity (US Patent 7,771,736, EP0001017636, issued in 2010) and is suspected of causing or promoting chronic botulism (*Shehata et al., 2012*). Glyphosate sorption efficiency onto biochar particles is both dependent on pH (high sorption at low pH; *Herath et al., 2016*) and the highest treatment temperature during biochar production (high sorption on high-temperature biochars; *Hall et al., 2018*). However, *Hall et al. (2018)* showed that glyphosate sorbed by biochar from pure water could be remobilized by adding 0.1M monopotassium phosphate solution. This finding indicates that biochar-sorbed glyphosate from feed may be remobilized in the digestive tract due to numerous ions potentially competing for sorption sites. Further research in vivo and/or in vitro in relevant matrixes is necessary, as low pH, for example, in the stomach, could favor glyphosate sorption (*Herath et al., 2016*). In a study with 380 dairy cows, *Gerlach et al. (2014)* showed that daily feeding with humic acids (120 g/day) or with a combination of 200 g of biochar and 500 g of sauerkraut juice for 4 weeks significantly reduced the glyphosate concentration in the urine of the cows that were fed with glyphosate contaminated silage.

Preliminary pesticide adsorption studies using biochar were already carried out in the 1970s (*Humphreys & Ironside, 1980*). Deposits of the systemic organophosphorus insecticide Runnel in the gastric mucosa of sheep were significantly reduced by the feeding 50 g of activated biochar per kg of feed, i.e., 5% amendment rate (*Smalley, Crookshank & Radeleff, 1971*). While it was reported that activated biochar was successfully used to adsorb pesticides in the digestive tracts of cattle, sheep and goats and were eventually excreted (*Wilson & Cook, 1970*), similar experiments in chickens did not show any significant effects on the residue levels in eggs and tissues (*Foster et al., 1972*). Feeding of biochar with Dieldrin contaminated feed, an organochloride insecticide that was widely used until the 1970s and is still persistent in the environment though it is banned now, resulted in a very significant reduction of the Dieldrin concentration in the fat of the pigs (*Dobson et al., 1971*). On the other hand, *Fries et al. (1970)* found no reduction in the levels of Dieldrin and DDT in milkfat when cows were fed one kg of activated biochar per day for 14 days. However, *Wilson et al. (1971)* found that when Dieldrin and DDT-contaminated feed was mixed with activated biochar at 900 g per animal and day, Dieldrin intake was reduced by 43% and DDT intake by 24%. When the contaminated feed and biochar were administered separately, DDT intake was not reduced as both the Dieldrin and DDT were probably absorbed by the oral mucosa already and not only in the digestive tract (*Fries et al., 1970*). Activated biochar also showed very good in vitro adsorption properties for the herbicide Paraquat (*Okonek et al., 1982*; *Gaudreault, Friedman & Lovejoy, 1985*), which has been banned in the EU since 2007 but is still legal in the US and other countries.

Fat-soluble organochlorine compounds such as Dibenzo-*p*-dioxin (PCDDs), Dibenzofuran (PCDFs) and dioxin-like PCBs are ubiquitous environmental toxins, and can often be detected in animal feed. These compounds accumulate in the adipose (fatty) tissue of animals and humans. Experiments with activated biochar to adsorb these substances were undertaken repeatedly in Japan (*Yoshimura et al., 1986*; *Takenaka, Morita & Takahashi, 1991*; *Takekoshi et al., 2005*; *Kamimura et al., 2009*). All experiments showed the strong affinity of the organochlorine compounds to activated biochar (*Iwakiri, Asano & Honda, 2007*). *Fujita et al. (2012)* carried out an extensive experiment with 24 laying hens whose feed contained the organochlorine compounds mentioned above and fed either with or without 0.5% biochar over a period of 30 weeks. Depending on the structure and aromaticity of the organochlorine compounds, concentrations of PCDDs/PCDFs, non-ortho PCBs and mono-ortho PCBs in the tissue and eggs of the laying hens could be reduced by more than 90%, 80% and 50%, respectively (*Fujita et al., 2012*). The fact that different organochlorine compounds are bound to different degrees by biochar has been previously demonstrated in studies of contaminated fish oil (*Kawashima et al., 2009*). In general, molecules with higher aromaticity have a stronger affinity to biochar; this also applies to polycyclic aromatic hydrocarbons (*Bucheli, Hilber & Schmidt, 2015*). *Olkkola & Neuvonen (1989)* concluded that the regular intake of biochar as food supplement can be very helpful in the elimination of industrial and environmental toxins including dioxins and PCB ingested by humans, a valid statement for animal feed too.

Detoxification of plant toxins

Another benefit of a regular use of biochar is the alleviation of adverse effects of naturally occurring though potentially harmful ingredients such as tannins contained in many feeds (*Struhsaker, Cooney & Siex, 1997*). Tannins are complex and extraordinarily diverse compounds that are partly beneficial but may also be harmful especially to ruminants. Tannins are often found in high protein feeds such as legumes and the strong taste repels the animals, which reduces digestability and weight gain (*Naumann et al., 2013*). Several studies have investigated how biochar feeding alters the impact of tannin-rich foods. *Van, Mui & Ledin (2006)* found that in goats, feeding 50–100 g of bamboo biochar per kg of a tannin-rich acacia leaf diet increased daily weight gain by 17% compared to the control without biochar. The authors found that digestion of crude proteins and nitrogen conversion were significantly improved. Apparently, there was an optimal biochar dose: While 50 and 100 g of bamboo biochar feed additions resulted in similar goat weight gains, feeding 150 g of the same biochar per kg diet did not show any improvement compared to control. *Struhsaker, Cooney & Siex (1997)* found, as previously described, that the consumption of wild fire derived charcoal by Zanzibar red colobus monkeys increased the nutritional efficiency of tannin-rich Indian almond and mango leaves. *Banner et al. (2000)*

found that the mixture of 10–25 g of activated biochar per day with rye significantly increased the uptake of tannin and terpene rich compounds. Similar results for sage and other terpenic and tannin-rich shrubs were reported by Rogosic et al. (2006, 2009), whereas others could not confirm that lambs consumed significantly more sage due to biochar amended feed (Villalba, Provenza & Banner, 2002).

In winter, when hardly any fresh pasture plants are available, sheep also eat bitterweed (*Hymenoxys odorata* DC.), which contains toxic levels of sesquiterpene lactones. Poage et al. (2006) conducted therefore a series of bitterweed feeding trials with 0.5–1.5 g of biochar per lamb per day mixed directly to the feed. While the lambs rejected the bitterweed-containing feed without biochar, they did consume bitterweed up to 26.4% of the total feed intake when combined with biochar revealing no signs of toxicosis.

Several studies have shown that poisoning of both livestock and sheep through contamination of feed with *Lantana camara, a species of flowering invasive species*, can be effectively treated with five g of biochar per kg of body weight (Pass & Stewart, 1984; McLennan & Amos, 1989). While five out of six calves recovered from *Lantana camara* poisoning after treatment with activated biochar, five out of six calves not treated with biochar died (McKenzie, 1991). Treatment with bentonite achieved similarly high cure rates, but complete healing took about twice as long. Similarly, significant results are found for treating Yellow tulip (*Moraea pallida*) poisoning of cattle (Snyman et al., 2009) and oleander poisoning of sheep (Tiwary, Poppenga & Puschner, 2009; Ozmaie, 2011).

Regular biochar feeding to improve performance and animal welfare

While therapeutic administration of biochar is a historically proven practice and has been scientifically studied for over 50 years and recommended as a cure for numerous symptoms, regular co-feeding of biochar with the purpose of improving productivity is discussed again only since 2010. The feeding of livestock with biochar and biochar products is rapidly spreading in practice, due to the apparently good experiences of farmers, especially in Germany, Switzerland, Austria and Australia. However, systematic scientific research on regular feeding with various types of biochar is still rare. One reason for this is the fact that with veterinary medicine and biochar research two areas of expertise collide that could hardly be more different and whose methods and vocabulary have little in common. The latter also explains why usually non-characterized or only poorly characterized biochar was used for feeding experiments.

Despite the diversity of biochar properties, key features of this heterogeneous material are similar and apparently lead to comparable effects when provided as feed supplement. The review of 27 peer reviewed scientific publications and clinical studies (Table 1) about regular biochar feeding revealed no negative effects on animal welfare and performance. Still, there are open question on some effects on long-term biochar feeding that should be addressed prior to an unconfined recommendation of regular biochar feeding. These include effects on the resorption of liposoluble feed ingredients and potential interaction with the mycotoxin fumonisin. These risks of regular biochar feeding are summarized in a separate section below. While results of feeding trials were sometimes neutral (no significant difference between biochar and control treatment), often one or

Table 1 Overview of published studies on biochar feeding.

Animal	Daily BC intake	Feedstock	HTT in °C	Activation	Blend	Weight increase in %	Duration in days	Other results and remarks	Source
Cattle	0.6% of feed DM	Rice hull	700	No		25	98	Reduced enteric methane emissions	*Leng, Inthapanya & Preston (2013)*
Bull	2% of feed DM	Wood	>600	No	Vitamin A	n.s.			*Kim & Kim (2005)*
Cattle	1% of feed DM	Rice husk	>600	No		15	56	15% feed conversion rate increase	*Phongphanith & Preston (2018)*
Goat	1% of body weight	Bamboo		No		20	84	DM, OM, CP digestibility and N retention increased	*Van, Mui & Ledin (2006)*
Goat	1% of feed DM			No		27	90	DM, OM, CP digestibility and N retention increased	*Silivong & Preston (2016)*
Pig	0.3% of feed DM	Bamboo	>600	Yes (900)	Bamboo vinegar	17.5	42	Improved the quality of marketable meat	*Chu et al. (2013c)*
Pig	0.3% of feed DM	Wood		No	Stevia	11		Higher meat quality and storage capacity	*Choi et al. (2012)*
Pig	1%, 3% and 5% of feed DM	Wood	450 °C	No	25% wood vinegar	n.s.	30	Increased duodenal villus height	*Mekbungwan, Yamauchi & Sakaida (2004)*
Pig	1% of DM feed	Wood	>600	No	Lactofermented	n.s.	28		*Kupper et al. (2015)*
Pig	1% of DM feed		>500			20.1	90	20.6% increased feed conversion rate	*Sivilai et al. (2018)*
Poultry	0.2% of DM feed	Wood		No		17	49		*Kana et al. (2010)*
Poultry	0.2% of DM feed	Maize cob		No		6	49	Improved carcass traits	*Kana et al. (2010)*
Poultry	2%, 4%, 8% of feed DM	Citrus wood		No		0	42	Heavier abdomen fat	*Bakr (2007)*
Poultry	2.5%, 5%, 10% of feed DM	Wood		No		0	42	Weight increase up to 28 days but not after 49 days	*Kutlu, Ünsal & Görgülü (2001)*
Poultry	0.3% of feed DM	Wood		No		3.9	140	Reduced mortality by 4%	*Majewska, Pyrek & Faruga (2002)*, *Majewska, Mikulski & Siwik (2009)*
Duck	1% of DM feed	Bamboo	>650	No	Bamboo vinegar	n.s.	49	Intestinal villus height increased	*Ruttanavut et al. (2009)*

(Continued)

Table 1 (continued).

Animal	Daily BC intake	Feedstock	HTT in °C	Activation	Blend	Weight increase in %	Duration in days	Other results and remarks	Source
Duck	1% of DM feed	Wood		No	Kelp	n.s.	21	Feed conversion rate increased	*Islam et al. (2014)*
Poultry	4% of DM feed	Woody green waste	550	No		n.s.	161	Egg weight increased by 5%; feed conversion ratio by 12%	*Prasai et al. (2016)*
Poultry	1% of DM feed	Rice husk	>550	No		n.s.		Reduced pathogenes in feces	*Hien et al. (2018)*
Poultry	0.7% of DM feed	Wood	>650	No	Lactofermented	n.s.	36		*Kupper et al. (2015)*
Poultry	1% of DM feed	Wood	>650	No	Lactofermented	5	37	Reduced foot pat and hook lesions by 92% and 74%	*Albiker & Zweifel (2019)*
Flounder	0.5% of DM feed	Bamboo		No		18	50	Feed and protein conversion rate increased	*Thu et al. (2010)*
Flounder	1.5% of DM feed	Wood		No	20% wood vinegar	11	56	Highest feed efficiency increase of 10% at 0.5% BC	*Yoo, Ji & Jeong (2007)*
Stripfish	1% of DM feed	Rice husk	>600	No		36	90	Significantly improved water quality	*Lan, Preston & Leng (2018)*
Stripfish	1% of DM feed	Wood		No		44	90	Significantly improved water quality	*Lan, Preston & Leng (2018)*
Carp	0.5%, 1%, 2%, 4% of DM feed	Bamboo		No		n.s.	63	Improved serum indicators	*Mabe et al. (2018)*
Stripfish	2% of feed DM	Bamboo		No	High VOC biochar	27	50	Survival rate increase by 9%	*Quaiyum et al. (2014)*
					Mean	9.9			

Note:
The table indicates the percentage weight increase of various livestock depending on the ingested biochar type and daily feed intake. A total of 61% of the 28 data set delivered weight increases while the remaining trials did not result in significant increases.

several of the following effects were observed when biochar was provided as feeding additive to livestock:

- Increase in feed intake
- Weight gain
- Increased feed efficiency
- Higher egg production and quality in poultry
- Strengthening of the immune system
- Improvement of meat quality
- Improvement of stable hygiene and odor pollution
- Reduction of claw and feet diseases
- Reduction of veterinary costs

Sorted by animal species, the following subsection reviews the scientific literature on medium to long term feeding of biochar in regard to improving livestock productivity, product quality, animal fitness, welfare and performance in the respective animal farming system. Risks of regular biochar feeding are summarized in a separate section.

Cattle

As evidenced by farmer practice, veterinary advice, and European regulations, biochar is already widely used as a regular feed supplement in cattle farming especially in Germany, Austria and Switzerland (European Biochar Certification body, Hans-Peter Schmidt, 2018, personal communication). However, there are only very few scientific studies on biochar feed additives for cattle so far.

Since 2011, the German veterinarian Achim Gerlach has been feeding 100–400 g of high temperature wood biochar (HTT 700 °C) per cow per day to numerous herds of cattle without detecting negative side effects (*Gerlach & Schmidt, 2012*; Hans-Peter Schmidt, 2018, personal communication). His survey of 21 farmers with at least 150 cattle revealed that overall health and vitality had improved since they had started biochar feeding. The somatic cell count (SCC) of the milk, an indicator of level of harmful bacteria, decreased significantly, whereas milk protein and milk fat content increased. When biochar additions to feed stopped, SCC quickly increased and a general loss of performance of the animals compared to the biochar-feeding period was observed. It was also reported that hoof problems were reduced, and that postpartum health was stabilized through biochar co-feeding. Within 1–2 days after the onset of the biochar feeding, diarrhea symptoms decreased and feces became firmer. Mortality rates declined, as did overall veterinary costs. The liquid manure viscosity improved significantly and the odor load of the manure decreased (*Gerlach & Schmidt, 2012*).

For 98 days, Leng, Preston & Inthapanya fed four cattle 0.6% of a rice hull-derived biochar, with another four in a control group without biochar in their feed. The biochar feeding resulted in a 25% higher weight gain compared to the control animals (*Leng, Preston & Inthapanya, 2013*). Another study, however, did not find any significant effect on weight gain and blood values in Hanwoo bulls when an undefined biochar was administered at a rather high dose of 2% (*Kim & Kim, 2005*). A supplement of 1% rice husk biochar was added to a basal diet consisting of ensiled cassava root, urea, rice straw and fresh cassava foliage (*Phongphanith & Preston, 2018*). Live weight gain increased by 15% and feed conversion rate also improved by 15% in the biochar treatment, compared to the control without biochar supplement. Interestingly, when a rice wine distillers' byproduct was added at 4% to the biochar-supplemented feed, the live weight gain and the feed conversion rate increased by 60% compared to the control without either supplement. They further found an increase of 18% compared to feeding with the rice wine distillers alone (without biochar), or 31% compared to the biochar-only supplement. This shows a strong interactive effect between the two supplements indicating that the combination and interaction of biochar with other feed additives should increasingly be investigated.

In a semi-continuous artificial rumen system, a high temperature biochar (HTT 600 °C) was added at 0%, 0.5%, 1% and 2% to a high-forage diet for 17 days. The biochar linearly increased the digestion of dry matter, organic matter, crude protein and fiber. Microbial protein synthesis also increased linearly. The microbial production of acetate, propionate and total volatile fatty acids in the artificial rumen increased (*Saleem et al., 2018*).

As early as 2010, Marc McHenry pointed to the possibility of using biochar as a feed additive not only to increase feed efficiency but to also increase nutrient availability of the manure, to protect ground and surface water, and to sequester carbon in the soil (*McHenry, 2010*). This cascading approach of improving not only animal performance and welfare but also various ecosystem services has been the subject of discussion and investigation by various authors since (*O'Toole et al., 2016*; *Schmidt & Shackley, 2016*; *Kammann et al., 2017*). A far-reaching study of these cascades has been carried out by *Joseph et al. (2015b)* in Australia: Since 2011, 60 grazing cattle on an Australian farm were fed 330 g per day of a high temperature biochar (HTT 600 °C) made from Jarrah wood mixed with 100 g of molasses. From 2011 to 2015, soil organic matter, pH ($CaCl_2$), Colwell-P, Colwell-K, electrical conductivity and the content of all exchangeable cations increased in the pasture soil that received the dung of the free ranging cattle. During its passage through the digestion system of the cattle, biochar seems to capture organic and mineral compounds with high plant fertilizing properties that would otherwise probably be subject to rather quick leaching during storage. Most of these captured plant nutrients (especially nitrogen and phosphorus) remain bound in the porous structure of the biochar until its incorporation into the soil, where they likely become, to a large extent, plant available as has also been found for biochar after aerobic composting (*Kammann et al., 2015*; *Schmidt et al., 2017*). The authors of the Australian study reported that increased retention of the digested nutrients in the biochar increased the fertilizing effect of the bovine manure so that no additional fertilizers was required for the pasture growth (*Joseph et al., 2015b*). However, they did not set-up a control pasture to proof the latter. To prove their conclusion, a more systematic scientific experiment would be required.

In addition to the improvement of the fertilizing properties of biochar-amended manure, the application of biochar to manure either via feed or via bedding materials is recommended as a potent strategy to reduce manure related greenhouse gas emissions (*Kammann et al., 2017*). When biochar (wood shavings, HTT 650 °C) was applied at 13% to a cattle slurry and subsequently applied to a field at 3.96 m^3 biochar ha^{-1}, the biochar decreased total NH_3-emissions by 77%, N_2O-emissions by 63% and CH_4-emissions by 100% compared to the control of cattle slurry only (*Brennan et al., 2015*).

Since 2012, German and Swiss farmers have been using biochar in the production of feed silage to stabilize lactic acid fermentation, prevent fermentation failure and reduce risks of fungal infestation and formation of mycotoxins (*O'Toole et al., 2016*). Lower levels of acetic acid and especially butyric acid are expected to minimize the risk of *Clostridia* infestation. The high-water holding capacity of biochar appears to buffer the water content of the silage, reducing the formation of excess fermentation liquids.

Calvelo Pereira et al. (2014) investigated the addition of various amounts and types of biochar (0–2.1–4.2–8.1–18.6% made from pine wood or maize straw and pyrolyzed at 350, and 550 °C, respectively) to hay silage and to cattle rumen liquid. The biochar treatments did not significantly affect the investigated silage quality parameters, nor did it negatively affect in vitro incubation with rumen fluid.

Goats and sheep

In a 12-week experiment with 42 young goats, it was found that feeding one g of bamboo biochar per kg of bodyweight resulted in significantly higher crude protein intake (*Van, Mui & Ledin, 2006*). The total amount of digested nitrogen increased and was thus lower in the urine and feces of the animals. The body weight increased on average 53 g per day compared to 44 g in the control group fed without biochar; a statistically significant difference of 20%. The basic feeding of the goats included a large proportion of tannin-rich acacia (*Acacia mangium*) leaves, and the authors hypothesized that biochar eased digestion of those leaves by sorption of their tannins which apparently lead to higher crude protein and improve total DM intake.

In a trial with groups of 12 goats ($N = 3$), growth performance was tested when a basal diet of tannin rich leaves of *Bauhinia acuminata* were provided either with or without 1% biochar (*Silivong & Preston, 2016*). Biochar improved the nutrient assimilation and led to a 27% increase in daily weight gain over the 100-day period of the trial. In another study, a goat feed additive of 1.5% and 3% activated coconut biochar did not produce significant improvement of feed intake nor did it alter the microbial community structure compared with the control (*Al-Kindi et al., 2017*). However, the activated biochar increased the fecal concentration of slowly decomposable carbohydrates while reducing fecal N. This left the authors to surmise a beneficial slow-down in the mineralization rate of the organic carbon contained in the manure when applied to soil, which may be beneficial for the built-up of soil organic matter.

Horses

Very few publications exist yet on feeding biochar to horses. *Edmunds et al. (2016)* investigated the effect of a woody biochar on the microbial community of the equine hindgut and the metabolites they produce. They did not find any significant effect of the biochar and concluded that the effect of biochar as a control for toxic substances is at its highest in the foregut or midgut of animals, and therefore should have little impact on the hindgut.

According to the EBC certified manufacturers of biochar and biochar products, horse breeders and farmers widely apply biochar in horse manure management and also in feeding, but apart from the above, not a single scientific study is known to the authors.

Pigs

Chu et al. published several fundamental studies in 2013 on the feeding of bamboo biochar to pigs. Young pigs ($N = 12$) were fed for 42 days in addition to their normal fattening diet (corn, wheat, soybean meal) either with 0%, 0.3% or 0.6% of biochar. The average weight gain during the trial period was 750 g per day in the control without biochar and 877 g per day in the 0.3% biochar treatment; this corresponded to a significant feed efficiency increase of 17.5%. Doubling the biochar supplement to 0.6% did not lead to statistically significant differences compared to the 0.3% treatment. While leucocytes, erythrocytes, hemoglobin, hematocrit and platelets did not differ significantly between the experimental groups, the biochar group showed significant positive effects on total protein, albumin, cholesterol, HDL-CH and LDL-cholesterol levels in the blood plasma. In addition, the cortisol content was significantly lower, which indicates a reduced susceptibility to stress (*Chu et al., 2013c*). In another study, the authors showed that feeding 0.3% and 0.6% bamboo biochar improved the quality of marketable meat and the composition of pig fat, with an increase in unsaturated fatty acid content and a decrease in saturated fat (*Chu et al., 2013b*). In a third study, the authors examined to what extent biochar feeding can replace the regular supplementation of growth-promoting antibiotics, something which is still legal in many though not all countries. In a very comprehensive publication (*Chu et al., 2013a*), they concluded that feeding 0.3% bamboo biochar gave the same growth rate in fattening pigs as the standard antibiotic treatment, notably without the negative side effects to the environment that antibiotics can have.

Another hog feed trial was done in South Korea using different concentrations of biochar and stevia mixed into the common diet of 420 pigs (*Choi et al., 2012*). While neither 30 g of biochar nor 30 g of stevia per kg of feed alone had any significant effects, 30 g of biochar plus 30 g of stevia had higher daily weight gain, feed efficiency and immune responses as well as significantly higher meat quality and storage capacity of meat products (*Lee et al., 2011*; *Choi et al., 2012*). In a Japanese study by *Mekbungwan, Yamauchi & Sakaida (2004)*, piglets were fed with increasing concentrations of a 4:1 mixture of a low temperature biochar (HTT 450°) and wood vinegar. When fed with 1%, 3% and 5% of this mixture, no statistically significant effects on body weight and feed efficiency were observed compared to the 0% control. However, duodenal villi height, an animal health indicator, increased significantly. The same authors showed 4 years later, with the same biochar-wood vinegar mix added at 1% and 3% to a protein-rich feed, that the biochar treatments prevented negative side-effects of pig fattening with protein-rich pigeon peas (*Mekbungwan et al., 2008*). The biochar-fed animals presented significantly better values in parameters related to health such as intestinal villi height, cell area and cell mitosis number compared to the control groups.

In Switzerland, Kupper et al. (2015) fed 80 weaned piglets for 28 days with a 1% commercial biochar feed additive mixture that had undergone a lactic fermentation beforehand. The biochar treatment did not reveal any significant difference in daily weight gain, feed consumption and feed conversion rate compared to the control group that received the same feed but without the biochar containing supplement. Moreover, no significant difference in NH_3-emissions of the stored or field applied manure was observed.

In a trial with native Moo Lath pigs ($N = 20$), the addition of 1% biochar to a basal diet consisting of ensiled banana pseudo stem and ensiled taro foliage increased the feed conversion rate by 10.6% compared to the control. The total weight gain of the piglets was on average higher by 20.1% ($p = 0.089$) after the 90 days of the experiment (Sivilai et al., 2018).

Poultry

Of all publications on the performance-enhancing use of biochar, a majority have focused on its use with poultry, not least because scientific studies using poultry are easier and less costly to perform than on large ruminants or pigs. One of the more frequently cited studies is that of Kana et al. (2010) who systematically fed two different biochars, one from corncobs and the other from canary tree (*Bakeridesia integerrima*) seeds, to broiler chickens at different feeding concentrations from 0% to 1% per kg feed. Unfortunately, the production of biochar was only designated as "traditional" and was not described in detail, but the high ash levels of 47% and 25%, respectively, indicate that a substantial portion of the initial biomass was burned and not fully pyrolyzed. Nevertheless, feeding both biochars up to 0.6% led to greater, mostly significant weight gain, while the higher dosages led to no further significant weight gain, but also to no weight loss compared to the control. Liver weight, abdominal fat nor bowel length and weight were affected by the biochar feeding. The study is an important indication that biochar derived from non-woody biomass and with a higher ash content may also be suitable for feeding, which is so far not allowed by the European Biochar Foundation (EBC) (2012). In a later study with the same biochars, the authors examined whether chickens can, thanks to the biochar supplement, be fed with 20% chickpeas, a feed that is protein-rich but generally difficult for chickens to digest. Surprisingly, when the ash-rich biochar from corncobs was added, the boiled chickpeas could be fed and provided the same weight gain in the broilers as the control without chickpeas. However, the lower-ash biochar from the tree seeds did not show the same effect here (Kana, Teguia & Fomekong, 2012).

Bakr (2007) used traditionally produced citrus wood charcoal purchased at the local market in Nablus and added them at very high dosages of 0%, 2%, 4% and 8% to the standard broiler feed. At 2%, significant increases on body weight, feed intake and feed efficiency were measured during the first three weeks compared to control. After this initial period, all results were similar. Of particular note in this study is that even the very high feeding dosage of 8% of a biochar of at least doubtful quality did not cause any adverse effects. Kutlu, Ünsal & Görgülü (2001) also used very high biochar dosages of up to 10%

of the base diet, and found that all dosages significantly increased basal feed intake in the first 28 days, and also weight gain and feed efficiency of both broilers and laying hens but did not show significantly higher gains after this initial period.

A Polish working group led by Teresa Majewska conducted several feed trials on chickens and turkeys between 2000 and 2012 (*Majewska & Pudyszak, 2011*; *Majewska, Mikulski & Siwik, 2009*; *Majewska, Pyrek & Faruga, 2002*). They achieved consistently positive results with doses of 0.3% of a hardwood biochar. They not only found higher weight gain and better feed efficiency, but also higher protein levels in the pectoral muscles and a significantly lower mortality compared to the control. Majewska et al. explained these improvements by (1) the detoxification of feed components, (2) the reduction in surface tension of the digestive pulp and (3) the improvement in fat loss in the liver.

Ruttanavut et al. (2009) did not find a statistically significant increase in duck growth when co-fed with a 1% biochar—wood vinegar blend, but they showed significant biochar effects on the size of the villi, the cell surface, and the rate of cell division in the gut, which confirms similar results from literature (*Samanya & Yamauchi, 2001*; *Ruttanawut, 2014*). *Islam et al. (2014)* showed in an experiment with 150 young ducks that feeding with 1% of a 1:1 mixture of biochar and sea tangle (*Laminaria japonica)* can be recommended as an alternative to the use of antibiotics in the feeding of ducks.

Several research groups have shown that the quality of chickens' meat can be significantly improved by feeding of biochar (*Cai, Jiang & He, 2011*; *Kim et al., 2011*; *Yamauchi, Ruttanavut & Takenoyama, 2010*; *Yamauchi et al., 2014*). It was for example found that no significant weight gain was recorded when fed with 0.5% activated coconut shell biochar but that Serum Glutamine, Oxaloacetic Transminase, Serum Glutamine Phosphate Transminase, Albumin and triglycerides as well as sensory evaluation and weight of abdominal fat, heart and spleen significantly improved while the cholesterol level decreased (*Jiya et al., 2013*, *2014*). Also, when broiler chickens were fed with 1% activated biochar the useful fatty acid, oleic acid and total mineral content of the meat increased significantly (*Park & Kim, 2001*). Other trials with 2% biochar or a mixture of bamboo biochar and wood vinegar did not show significant differences in meat quality compared to controls (*Sung et al., 2006*; *Fanchiotti et al., 2010*; *Ruttanawut, 2014*).

It was observed in several studies that the strength of eggshells can be improved by co-feeding biochar (*Kutlu, Ünsal & Görgülü, 2001*; *Ayanwale, Lanko & Kudu, 2006*; *Kim et al., 2006*). *Yamauchi, Ruttanavut & Takenoyama (2010)* found an increase in egg production of nearly 5% when hens were fed with a blend of bamboo biochar and wood vinegar. The collagen content of the eggs increased highly significantly by 33% with a 1% feed of the same bamboo biochar—wood vinegar mixture. Collagen not only increases the shelf life of the eggs but is also an interesting ingredient for pharmaceuticals and cosmetics (*Yamauchi et al., 2013*).

Prasai et al. (2016) investigated biochar, bentonite and zeolite for selective pathogen control in hens. Their treatments involved the commercial layer diet (control group) amended with biochar, bentonite and zeolite at 4% w/w, respectively. While bird weight and number of eggs did not differ significantly between the control and the biochar treatment, the total egg weight increased by 5% and the feed conversion ratio increased by

12% compared to the control. Feeding bentonite and zeolite revealed comparable increases and non-significant differences to biochar, respectively. The biochar feed amendment did not result in altered gut microbial community richness and diversity compared to the control. However, individual phylotypes at different phylogenetic levels did respond differently to the three amendments and reduced especially the abundance of *Helicobacter* and *Campylobacter*. Both genera are gram-negative and include multiple pathogenic species. The authors demonstrated that biochar, bentonite and zeolite can be used to selectively reduce the abundance of some major poultry zoonotic pathogens without reducing chicken microbiota diversity or causing major shifts in the gut microbial community and are thus a viable alternative to antibiotics in the poultry industry. A recent Vietnamese study on supplementing chicken feed with 1% rice husk biochar confirmed positive effects on pathogen occurrence with reduced plasma triglycerides, total coliform bacteria in litter and *E. coli* in feces (*Hien et al., 2018*). However, no impact on live weight gain, feed consumption and feed conversion ratio were observed.

In Switzerland, two groups of 400 broilers were fed for 36 days with a 0.7% biochar supplement provided as a commercial feed additive mixture that had undergone a lactic fermentation beforehand (*Kupper et al., 2015*). The biochar treatment did not reveal any significant difference in daily weight gain, feed consumption, feed conversion rate or food pat and hook lesions compared to the two control groups that received the same feed without the biochar containing supplement. Moreover, no significant difference in NH_3-emissions of the stored or field applied broiler manure was measured. The results of *Kupper et al. (2015)* are in puzzling contradiction with a similar trial in the same country undertaken at the Swiss Aviforum where groups of 270 broilers with four replicates were fed for 37 days with the same 0.9% biochar based commercial feed additive, with 1% pure wood based biochar (HTT of 700 °C) or with 0% biochar as control group (*Albiker & Zweifel, 2019*). Here, the weight gain increased significantly by 5% (fermented biochar product) and 6% (pure biochar) compared to the control. Moreover, both biochar treatments decreased the foot pat and hook lesions by 92% and 74%, respectively, compared to the control.

For a study at West Virginia University with test groups of 1,472 broiler chicks ($N = 8$), pyrolyzed poultry manure was provided as feed additive despite insufficient feed quality analyses (*Evans, Boney & Moritz, 2016*). The arsenic content of the poultry manure biochar exceeded the threshold of the European Biochar Feed Certificate (*European Biochar Foundation (EBC), 2018*) by a factor of 6.5, and no PAH analyses were carried out, despite using gasification technology that is known for the risk of producing biochars with high levels of PAH contaminations which often exceed threshold values of the EBC by factor 100 and more (*Hilber et al., 2012*; *Bucheli, Hilber & Schmidt, 2015*). Irrespective of these issues, supplementing poultry manure biochar at 2% increased the feed conversion ratio by 7% while at 4% biochar supplementation the life weight gain decreased by 8% both compared to the control. No other investigated parameter showed significant differences to the control over the 21-day experimental period. The feeding of such pyrolyzed material is in several regards not in agreement with the EBC-feed standard, and feeding uncharacterized excrement-based materials is certainly not up to ethical standards.

In an Australian trial, groups of 20 layer hens ($N = 4$) were fed a biochar made at 550 °C from green wood waste at rates of 0%, 1%, 2% and 4%, respectively (*Prasai et al., 2018a*) for 25 weeks. While no significant difference in weight gain was observed, the feed conversion ratio improved significantly between 10% and 13% in the three biochar treatments compared to the control without biochar. The egg weight was 5% higher in the 2% biochar treatment and 4% higher in the 4% treatment compared to the control. Standardized indicators of egg quality (i.e., Haugh unit, Albumen height, stability of egg shell) where not changed by the biochar feed amendment. The Yolk color index, however, decreased with increasing biochar dosage. The same effect was also found when bentonite or zeolite was used instead of biochar. Yolk color is mainly the result of carotenoid content (*Bovšková, Míková & Panovská, 2014*). Carotenoids are lipophilic organic molecules that accumulated from the feed. Thus, we hypothesize that biochar may sorb a certain amount of lipophilic ingredients of the feed. The N-balance between feed-N intake, egg-N, excreta-N and lost N did not differ significantly between the treatments though the excreta-N was reduced by 20–34% in the 2% and 4% biochar treatment compared to the control. The lower recovery of N in excreta is indicative of a more efficient digestive extraction of N, consistent with the observed higher feed conversion efficiency. Remarkably, the inclusion of 2% and 4% biochar maintained egg production at normal levels when birds were challenged with fungal-contaminated feed. In the control treatment, the contaminated feed led to decreased egg production by 16%. The same main author found, in another publication based on a similar trial with the same 1%, 2% and 4% biochar amendments, improvements of the poultry manure especially in regard to granule size, water retention and decomposition characteristics (*Prasai et al., 2018b*). N-contents in the decomposed poultry manure were lower by 20% and 26%, respectively, in the treatment with 2% and 4% biochar feed compared to the control. NH_3-emissions of the manure, measured in a separate experiment using incubated bell jars, increased by 31% in the treatments with 2% and 4% but not with 1% biochar feed amendments compared to the control. This increase in ammonia emissions due to high doses of poultry feed applied biochar is puzzling as the addition of higher dosages (5–15% (m/m)) of biochar to poultry manure composting was shown to decrease ammonia emissions between 53% and 89% (*Rong et al., 2019*). Apparently, biochar affects poultry manure composting differently when applied to the feed versus when applied directly to the manure.

Aquaculture

Nowadays aquaculture provides as much product for human consumption as capture fisheries, yet it causes considerable harm to the environment if effluents with fish feces and excess feed nutrients are not treated and recycled into valuable fertilizers (*UN, 2016*). Biochar supplements have been fed to fish with the intention to improve water quality as well as fish health and productivity. Japanese flounder were fed with 0–4% incremental doses of a bamboo biochar mixed into the regular feed (*Thu et al., 2010*). While all biochar feed additions resulted in significantly higher flounder weight gains, the variability of individual results was so high that only the 0.5% dose provided statistically significantly higher weight gain rates of 18%. It was noteworthy that all biochar feeding rates resulted in significantly lower nitrogen excretions and reduced the nitrate content in the fish water by >50%. In a South Korean experiment also with flounder, dosages from 0% to 2% of a biochar—wood vinegar blend were fed. At a dose of 1%, the feed efficiency increased significantly by 10%, and also the total weight gain of the fish was significantly higher (*Yoo, Ji & Jeong, 2007*). The authors concluded that feeding rates between 0.5% and 1% of DM feed intake may deliver maximum weight gain and feed efficiency.

Two different biochars, one made from rice husks in a TLUD stove (*Anderson, Reed & Wever, 2007*) and one made from wood in traditional charcoal kilns, were compared as a 1% feed additive for tank raised striped catfish (*Pangasius hypophthalmus)* (*Lan, Preston & Leng, 2018*). Growth rates increased by 36% with the rice husk biochar and 44% with the wood biochar compared to the control. Both biochars led to 25% increased ratio of weight to length indicating an enhanced flesh to bone ratio due to the faster growth rate caused by the biochar additive. Water quality improved significantly as levels of ammonia nitrogen, nitrite, phosphate and chemical oxygen demand decreased by 24%, 22%, 15%, 21%, respectively, in the rice husk biochar treatment with similar values for the other biochar. The authors hypothesized that biochar may facilitate the formation of biofilms as habitat for gut microbiota which could be the explanation for the improved growth rates.

In China, a dietary bamboo biochar was added to the feed of juvenile common carps at rates from 1% to 4% (*Mabe et al., 2018*). The biochar treatments did not produce any obvious effect on the growth performance of the carps compared to 0% control. However, significant improvements were reported on serum indicators such as alanine aminotransferase, aspartate aminotransferase, total protein, triglycerides, total cholesterol, high density lipoprotein (HDL) and glucose, demonstrating an increase in fish quality and health. The most beneficial effects were found at the highest biochar dosage. No adverse effects were observed.

Reduction of methane emissions from ruminants

Ruminant production accounts for about 81% of the total GHG from the livestock sector (*Hristov et al., 2013*). While in chickens, pigs, fish and other omnivores most of the greenhouse gas emissions are caused by the decomposition of solid and liquid excretions, ruminants' GHG emissions are mainly produced by direct gaseous excretions through flatulence and burping (eructation). The latter mainly affects cattle which are capable of producing 200–500 l of methane per day (*Johnson & Johnson, 1995*). These methane emissions, mainly produced through rumen microbial methanogenesis, are responsible for 90% of the GHG caused by cattle (*Tapio et al., 2017*).

In the bovine rumen, methanogenesis is carried out by archaea that convert microbial digestion products H_2 and CO_2 or formate (HCOOH, methanoate) to CH_4 to gain energy under anoxic conditions. While hydrogen serves as an electron donor for the microbial reduction of CO_2 to methane (CH_4), the reduction of formate (requiring six electrons to be reduced to H_2 and CO_2) can have several biochemical pathways. The production of methane means a significant loss of energy for the animal (from 2% to 12% of the total energy intake; *Tapio et al., 2017*) as the high-energy methane cannot be digested any further and has to be eliminated almost entirely through eructation (burp) and only minimally via flatulence from the digestive tract (*Murray, Bryant & Leng, 1976*). Since methane is a 28–34 times more harmful than CO_2 (global warming potential with and without climate-carbon feedbacks over a period of 100 years; *Myrhe et al., 2013*), there is an increasing interest in feed supplements that not only increase feed efficiency, but also can reduce methane emissions resulting from ruminant digestion.

Numerous studies have sought to find other electron acceptors besides CO_2 and enteric fatty acids to reduce methanogenesis. However, until recently, apart from the addition of nitrate and sulfate reacting to ammonia and hydrogen sulfide, respectively, which are toxic for the animals in higher concentrations, no convincing options have been found to date (*Van Zijderveld et al., 2010*; *Lee & Beauchemin, 2014*).

The first evidence that biochar might act as an electron acceptor and reduce methane production in the rumen came from Vietnam in 2012 (*Leng, Inthapanya & Preston, 2012*; *Leng, Preston & Inthapanya, 2012*). In vitro studies revealed that 0.5% and 1% biochar additions to the ruminal liquid significantly reduced methane production by 10% and 12.7%, respectively. Higher levels of biochar did not further reduce methane production. All experiments were conducted in the presence of 2% urea as a non-protein source of nitrogen. When urea was replaced with nitrate (6% of DM feed intake as KNO_3 to supply the same amount of N), methane production decreased by up to 49%.

While both, nitrate and biochar, may act as electron acceptor in the rumen and likely explain at least part of the effect, it is difficult to elucidate on the base of the data provided why the methane reductions by nitrate (–29%) and biochar (–22%) were higher when fed combined (–49%). However, as the effect appears dosage independent (0.5% or 1% biochar) it is unlikely that the two substances reduce methane production by the same mechanisms. It may be hypothesized that the biochar acts as a redox-active electron mediator that takes up electrons from microbial oxidation reactions (e.g., oxidation of acetate to CO_2) and donates the electron at a certain distance from the microbial reaction center (at another spot of the same biochar particle) to mediate an abiotic reduction of nitrate (*Saquing, Yu & Chiu, 2016*). Biochar at feeding ratios of about 1% (100 g/day) would not have the capacity to act as terminal electron acceptor for all rumen produced hydrogen considering a daily production of about 200 l methane for the various studies of *Leng, Inthapanya & Preston (2012)* in SE-Asia and up to 500 l methane for typical cattle in Europe or the US. Nitrate (at 6% of DM intake) would have this capacity as terminal electron acceptor but is not efficient as direct electron acceptor in microbial oxidation reaction due to the toxic effects of its reaction products (i.e., nitrite and ammonia).

Another likely mechanism is the biotic reduction of nitrate through Methylomirabilis oxyfera-like bacteria using the supplemented nitrate as an oxygen source for methane oxidation in the rumen. Denitrifying anaerobic methane oxidizing (DAMO) bacteria like *Candidatus* Methylomirabilis oxyfera belonging to the NC10 phylum were shown to efficiently oxidize methane anaerobically in deep lake sediments (*Deutzmann et al., 2014*). NC10 DAMO bacteria were equally found in wetlands (*Shen et al., 2015*), in grassland soils used for animal husbandry (*Bannert et al., 2012*), and with a robust abundance of 3.8×10^5 to 6.1×10^6 copies g^{-1} (dry weight) in flooded paddy fields (*Shen et al., 2014*). DAMO bacteria were further found in the rumen fluid of Xinong Saanen dairy goats in Southern China. The proportion of NC10 in total bacteria in the rumen fluid was 10%, and it could clearly be seen that NC10 mediated nitrate reduction led to reduced enteric methane emissions (*Shen et al., 2016*). Notwithstanding further evidence, it may be hypothesized that the additional effect of combined biochar and nitrate supplements is due to the biotic denitrifying methane oxidation that might further be enhanced through electron accepting and redox mediating properties of the biochar. Systematic investigations to better understand the likely mechanisms are urgently needed.

In vivo experiments showed that methane formation in cattle could be reduced by 20% when 0.6% of biochar was added to the ordinary compound feed (*Leng, Preston & Inthapanya, 2013*). When the same amount of biochar was combined with 6% potassium nitrate, methane emissions decreased by as much as 40%. In addition to reducing methane emissions, highly significant bovine weight gain (+25%) was observed in the experiment as compared to the control, suggesting an increase in feed efficiency and/or reduced energy conversion losses. The biochar in this and the earlier in vitro trial was produced at high temperatures (HTT = 900 °C) from silicon-rich rice husks, which suggests a high electrical conductivity and electron buffering capacity (*Yu et al., 2015*; *Sun et al., 2017*) which may lead to greater efficiency of fodder-decomposing redox reactions. *Leng, Inthapanya & Preston (2013)* have further shown that different biochars have different effects on methane emissions. A likely reason for this are differences in electrical conductivity and in electron buffering (*Sun et al., 2017*) depending on the biomass and pyrolysis temperature, which determine the biochar's properties of transmitting electrons between different bacterial species.

Leng, Inthapanya & Preston also examined the rumen fluid of cattle previously fed with and without biochar. They found that rumen fluid from cows that had been fed biochar produced less methane than rumen fluid from non-biochar-fed cattle. This suggests that the animals fed biochar may have had a different microbial community in the rumen (*Leng, Inthapanya & Preston, 2012*). *Phanthavong et al. (2015)* also found a significant decrease in methane emissions over a 24-h period in in vitro tests with 1% biochar added to a manioc root feed mix, but only by about 7%.

In 2012, a Danish team of researchers led by Hanne Hansen published the results of an in vitro study with large doses of various, but poorly characterized biochars and their effects on methane production of rumen fluids (*Hansen, Storm & Sell, 2012*). All tested biochars (made from wood or straw with slow pyrolysis or gasification) tended (p = 0.09) to reduce methane emissions from 11% to 17%, with an activated biochar showing the highest reduction rate. However, the enormously high addition of 9% cannot be considered as viable as this would surely impact feed digestibility on the long term. *Winders et al. (2019)* did not detect any significant reductions on methane emissions in steers over a 23 h period when using the more realistic biochar supplement rates of 0.8% and 3%.

Four biochars (from pine wood chips and corn stover, each pyrolyzed at 350 and 550 °C) were co-fermented at rates of 0.5%, 1%, 2% and 5% in ryegrass silage and used as feed substrates in an in vitro trial with rumen liquid (*Calvelo Pereira et al., 2014*). None of the biochar treatments revealed any effect on methane production as compared to the control.

Due to the promising results of *Leng, Inthapanya & Preston (2012)* several other research groups have carried out in vitro experiments though without obtaining significant results which, therefore, where not published (Belgium, USA and Germany, Hans-Peter Schmidt, 2018, personal communications). Until today, only the research group of Ron Leng were able to produce and reproduce high reduction rates of methane production both in vitro and in vivo. It is impossible yet to identify a convincing reason or mechanism to explain the strong divergence of the results. It might be due to the particular 900° gasifier rice-husk biochar or to the non-common feed used in their trials (tannin rich cassava roots and foliage that may provide terminal electron acceptors) or the particular rumen microbiota of the South-East Asian cattle that may contain higher rates of DAMO bacteria. The experiments from Europe, New Zealand and America with conventional cattle fodder and standard biochar prudently suggested, that biochar alone (i.e., without nitrate as oxygen source or terminal electron acceptor) may not live up to the expectations to reduce enteric methane emission of cattle (Table 2).

This conclusion is confirmed by a recent and perhaps the most systematic and complete in vitro study to date, at the University of Edinburgh (*Cabeza et al., 2018*). The authors investigated the effects on in vitro rumen gas production and fermentation characteristics of two different rates of biochar (10 and 100 g biochar/kg substrate, i.e., 1% and 10%) made at two different temperatures (HTT 550 or 700 °C) and from five different biomass sources (miscanthus straw, oil seed rape straw, rice husk, soft wood pellets and wheat straw). The methane production was reduced by all biochar treatments and at both concentrations levels by about 5% compared to the control without biochar. There was no significant difference between the different types and amounts of biochar. The absence of significant differences between those very different biochars is puzzling though an important milestone towards the understanding of biochar's mechanisms in animal digestions because there has to be a common cause leading to the same effect between all these different biochars.

A new perspective on the subject was recently put forth by *Saleem et al. (2018)* who used an artificial semi-continuous rumen system to test the effect of a high temperature biochar that was post-pyrolytically treated to acidify the biochar to a pH of 4.8. For a high-forage based diet, 0.5%, 1% and 2% of this acidic biochar reduced methane production by 34%, 16% and 22%, respectively. All other biochars in all of the experiments reviewed here were alkaline (pH between 8 and 11.5). The acidification of biochar not only oxidizes the carbonaceous surfaces and makes the biochar hydrophilic, it also modifies the redox behavior and thus its "affinity" for microbial interaction. As this is, to our knowledge, the first and only experiment to demonstrate a reduction of methane emissions using acidified biochar and as there are no systematic investigations about the acidification effect yet, it is too early to draw a definitive conclusion. However, it is an indication that post-pyrolytic treatment of biochar has the potential to design and optimize the biochar effects in animal digestion, and, notably, to reduce enteric methane emissions.

Table 2 Overview of published studies about biochar effects on enteric methane emissions.

Daily BC intake/content of rumen liquid	Type of trial	Feedstock	HTT in °C	Activation	Blend	CH_4-reduction	Source
0.5% to ruminal liquid	In vitro	Rice husk	900	No	2% urea	10%	*Leng, Inthapanya & Preston (2012)*
1% to ruminal liquid	In vitro	Rice husk	900	No	2% urea	12.7%	*Leng, Inthapanya & Preston (2012)*
1% to ruminal liquid	In vitro	Rice husk	900	No	6% KNO_3	49%	*Leng, Inthapanya & Preston (2012)*
0.6% of feed DM	In vivo	Rice husk	900	No		20%	*Leng, Preston & Inthapanya (2013)*
0.6% of feed DM	In vivo	Rice husk	900	No	6% KNO_3	40%	*Leng, Preston & Inthapanya (2013)*
1% of feed DM	In vivo	Rice husk	900	No	Manioc root feed	7%	*Phanthavong et al. (2015)*
9% to ruminal liquid	In vitro	Wood/straw		Partly		n.s. (11–17%)	*Hansen, Storm & Sell (2012)*
1% of DM feed	In vivo	Wood	>600			n.s.	*Winders et al. (2019)*
0.5%, 1%, 2%, 5% of rumen incubation	In vitro	Wood/corn stover	350/550	Ensiled	Mixed to ryegrass before ensiling	n.s.	*Calvelo Pereira et al. (2014)*
1%, 10% of DM feed	In vitro	Miscanthus straw/ oil seed rape straw/rice husk/ soft wood pellets/ wheat straw	550/700	No		5%	*Cabeza et al. (2018)*
0.5%, 1%, 2% of DM feed	In vitro	pine	400–600	Acidification to pH 4.8		34%, 16%, 22%	*Saleem et al. (2018)*

Note:
The table indicates the reductions of enteric methane emissions of cattle due to biochar feed supplements or additions to rumen liquids summarizing biochar dosages, pyrolysis feedstock and temperature and post-pyrolytic treatments.

The promising results of *Leng, Inthapanya & Preston (2012)* when feeding biochar in combination with nitrate call for systematic investigations of (1) pyrolytic and post pyrolytic treatments (e.g., pyrolysis temperature, activation, acidification), (2) feed blending with terminal electron acceptors (e.g., nitrate, urea and humic substances; *Md Shaiful Islam et al., 2005*), (3) co-feeding with oxygen sources for anaerobic methane oxidation (nitrate) and (4) inoculation with Methylomirabilis oxyfera-like bacteria to oxidize methane.

Possible side effects of biochar

Based on the literature compiled in the present review, none of the activated and non-activated biochars used as feed additive or veterinary treatment had toxic or negative effects on animals or the environment. No negative side effects were reported either in short-term or long-term administration trials.

There are a growing number of farmers that have been feeding their livestock with biochar additives on a daily basis for several years without noticing negative side-effects (*Kammann et al., 2017*; C. Kammann et al., 2017, personal communications).

However, there are only very few if any long term biochar feeding trials with clinical follow-up (*Struhsaker, Cooney & Siex, 1997*; *Joseph et al., 2015b*). In the absence of clinical long-term feeding trials with biochar, long-term experiments with oral administration of activated carbon to humans seem to indicate rather low risks. The administration of 20–50 g activated biochar daily in uremia patients for 4–20 months did not produce significant side effects (*Yatzidis, 1972*). *Olkkola & Neuvonen (1989)* maintained dosages of 10–20 g administered three times a day over a period of several months in human patients without negative side effects.

The main risks of long-term biochar feeding may arise (1) from shifting microbial species composition in the digestion system (microbiome) and (2) from the potential adsorption of essential feed compounds and/or drugs. Only a few scattered studies have addressed both points.

With regard to the microbiome, the adsorptive capacity of activated biochar for the beneficial bacterial flora in the digestive tract of dairy cows was examined using gram-positive *Enterococcus faecium*, *Bifidobacterium thermophilum* and *Lactobacillus acidophilus* (*Naka et al., 2001*). Although activated biochar certainly adsorbs strains of the normal, healthy bacterial flora too, adsorption of these bacterial strains was significantly lower than the adsorption of the dangerous *E. coli* O157: H7 strain, which is gram-negative. Biochar appeared to positively affect the ratio of (certain) beneficial bacterial flora to (certain) pathogenic flora. However, it must be systematically investigated and mechanistically understood for a much larger number of digestive and pathogenic microorganisms, before a more general conclusion can be drawn. Our review suggests that the impact of biochar on microorganisms depends on the cell envelope, that is, the gram-stain with gram-positive (plasma membrane plus 20–80 nm of peptidoglycan) not being or being less well sorbed to biochar, while gram-negative bacteria (plasma membrane plus 10 nm peptidoglycan plus outer membrane) are better sorbed. However, the structure of the cell envelope and the fact of being gram-positive or negative does not, on its own, indicate whether a bacteria is a pathogen or not.

The potentially selective action of biochars on various bacterial genera opens up the possibility of inoculating the biochar as a carrier matrix with beneficial bacteria, for example, to administer gram-positive *Lactobacilli*. to positively influence the intestinal flora (*Naka et al., 2001*). Different groups of authors have found that pathogens are generally bound more strongly than the native intestinal flora to biochar in the digestive tract (*Naka et al., 2001*; *Watarai, Tana & Koiwa, 2008*; *Choi et al., 2009*; *Chu et al., 2013a*). The hypotheses put forward indicate a possible correlation with more favorable pore size distribution for the adsorption of pathogens, as well as the observation of the (nonspecific) promotion of beneficial microorganisms such as *Lactobacilli*. This combination could positively target the digestive milieu and suppress pathogens.

With regard to sorption, biochar can work against human poisoning and drug overdose (*Park, 1986*), but thus could also counteract intended benefits of drugs. Based on our review, the same can be proclaimed regarding pharmaceuticals used to treat livestock. It is evident that acute, temporary treatment and continuous addition to feed over years do not underlie the same risk assessment. *Fujita et al. (2012)* conducted a comprehensive study in 2011, where they examined the influence of biochar feeding on hens' health and egg quality. Histopathological studies showed no changes in the digestive tract or in the liver. Examination of the egg yolk showed that fat-soluble vitamins A and D3 did not show a statistically significant trend towards lower concentrations, but that the vitamin E content in the eggs was reduced by about 40% when hens were fed daily with 0.5% biochar (*Fujita et al., 2012*). Although all other quality parameters such as fatty acids, oxidative stability and mineral content in the eggs were not affected by biochar feeding, it was the first evidence that a beneficial compound like a vitamin can be significantly reduced by co-feeding biochar. The above mentioned reduction of carotenoids in egg yolks indicated by changes in yolk color (*Prasai et al., 2018a*) further supports the conclusion that systematic research with well-defined biochars and a focus on liposoluble feed ingredients like vitamin E and carotenoids is needed before industrial scale-up of long-term biochar co-feeding can be safely recommended. However, compared to a large spectrum of other feed additives and ubiquitous pesticide and mycotoxin contamination of animal feed, risks of quality-controlled biochar feed can be considered low, even when supplemented on a regular basis.

Administration of biochar feed and biochar quality control

Biochar should not be fed without complete biochar analysis and control of all relevant parameters of current feed regulations such as provided by the European Biochar Feed Certificate (*European Biochar Foundation (EBC), 2018*). The analysis should be carried out by an accredited laboratory specialized in biochar and feed analytics. In addition, as required by the EBC, biochar should always be processed and administered moist to avoid the formation of dust (*European Biochar Foundation (EBC), 2012*). If this is respected, biochar can be added to all common feed mixes and is usually mixable with all common feeds. Feed quality biochar may also be added to animal drinking water and, in the case of acute intoxication, activated biochar should be administered in aqueous suspension (*Neuvonen & Olkkola, 1988*). Depending on livestock species, the biochar may also be provided in freely accessible troughs on the pasture or in the stable, without previous mixing into daily feed. Often, the biochar is mixed with popular supplements such as molasses (*Joseph et al., 2015b*) or flavoring such as saccharin, sucrose and the like (*Cooney & Roach, 1979*). Some German and Swiss farmers inject 1% (vol) of biochar into silage towers or silage bales via automated equipment (*O'Toole et al., 2016*).

In many of the experiments cited here, biochar was not administered alone, but in admixture with other functional feed supplements such as humic acid, wood vinegar, sauerkraut juice, eubiotic liquids, stevia, nitrate or tannins, the effect of the mixture often being greater than with separate feeding of the individual components. Those combinations of biochar with various other feed supplements open a huge scope for further research and the reasonable expectation that suitable feed mixtures can be developed for specific purposes and animal species.

The adsorption capacity of biochar depends in particular on the specific surface area, surface charge and the pore size distribution. Activation of biochar significantly increases the specific surface area (from approx. 300 m^2 to >900 m^2), but the increase in surface area is mainly due to the opening of micropores (<2 nm). These micropores are mostly too small for the higher molecular weight substances or bacterial pathogens relevant for animal digestion. *Galvano et al. (1996b)* found that biochar with dominating micro porosity (<2 nm) had lower adsorption capacities for mycotoxins due to slow diffusion of these toxins into the pore-system. This was also the case for other investigated toxic compounds such as pesticides, PCBs, dioxins or pathogens, as was demonstrated by *Edrington et al. (1997)* when highly activated biochar did not reduce the toxic effects of aflatoxin in chickens more strongly than non-activated biochar. Therefore, the activation of biochar may not significantly increase the specific adsorption capacity for certain target substances or organisms. To produce a biochar with a particularly high content of accessible meso and macro pores, downstream activation is not necessary and can be achieved merely by adjusting the pyrolysis parameters. Generally speaking, a higher meso-porosity is achieved at pyrolysis temperatures above 600 °C (*Brewer et al., 2014*).

Depending on the activation method, biochar activation and acidification can greatly modify the electron (and proton) mediating capacity (*Chen & McCreery, 1996*), however, to date no systematic research has been done with such modified biochars in animal feeding. Currently, only pyrolysis temperature was identified as main driver for the redox behavior, revealing temperatures between 600 and 800°C as optimal (*Sun et al., 2017*).

To minimize condensate deposition on biochar surfaces and to ensure that PAH contents stay below common thresholds (*European Biochar Foundation (EBC), 2012*) sufficient active degassing of the cooling biochar at the end of the pyrolysis process is mandatory, for example, by using inert gas or by sufficient counter flow ventilation during discharge (*Bucheli, Hilber & Schmidt, 2015*).

Biochars used in the various studies were mainly derived from wood, but also from coconut shells (*Jiya et al., 2013*), rice husk (*Leng, Preston & Inthapanya, 2013*), shea butter stocks (*Ayanwale, Lanko & Kudu, 2006*), bamboo (*Van, Mui & Ledin, 2006*; *Chu et al., 2013a*), corn stover (*Calvelo Pereira et al., 2014*), corncob (*Kana et al., 2011*), straw (*Cabeza et al., 2018*) and many other types of biomass. According to current publications, there is no scientific basis to prefer one source of biomass over another to produce feed-grade biochar. As long as important guidelines for the H/C_{org} ratio (= degree of carbonization), carbon and heavy metal contents, PAHs and other organic pollutants are met, biochar from woody as well as non-woody precursors may safely be used for co-feeding purposes.

The European Biochar Certificate (EBC), a voluntary industry standard, has been controlling and certifying the quality of biochar for use in animal feed since January 2016 (*European Biochar Foundation (EBC), 2018*). To date, six biochar producing companies have obtained the EBC-feed certificate (*European Biochar Foundation (EBC), 2013*). The EBC Feed Certificate guarantees compliance with all feed limits prescribed by the EU regulations and, moreover, certifies sustainable, climate friendly production (*European Biochar Foundation (EBC), 2018*).

CONCLUSIONS

The use of biochar as a feed additive has the potential to improve animal health, feed efficiency and livestock productivity, to reduce nutrient losses and greenhouse gas emissions and to increase manure quality and thus soil fertility. In combination with other good farmer practices, biochar could improve the overall sustainability of animal husbandry. The analysis of 112 scientific papers on biochar feed supplements has shown that in most studies and for all farm animal species, positive effects on different parameters such as growth, digestion, feed efficiency, toxin adsorption, blood levels, meat quality and/or emissions could be found. However, a relevant part of the studies obtained results that were not statistically significant. Most importantly, no significant negative effects on animal health were found in any of the reviewed publications.

It is undeniable that, despite the large number of scientific publications, further research is urgently needed to unravel the mechanisms underlying the observed results and to optimize biochar-based feed products. This applies in particular to the characterization of the biochar itself, which in the majority of studies was insufficiently analyzed. The electrochemical interaction of biochar and organic systems is extremely complex and needs considerable more fundamental research and systematic in vivo trials. Moreover, if biochar's role within animal digestion is mainly to act as a mediator and carrier substance, the combination with other feed additives and inoculants may be mandatory to achieve the full functionality of biochar for its beneficial use in animal digestion and animal health.

Based on the scientific literature published so far, it can be concluded that (1) a general efficacy of biochar as feed supplement can be observed and (2) biochar feeding can be considered safe at least for feeding periods of several months. Despite this positive assessment, regular feeding of biochar should never induce livestock farmers to compromise on the quality of feed and animal welfare standards.

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Competing Interests

The authors declare that they have no competing interests. Hans-Peter Schmidt and Nikolas Hagemann are employed by Carbon Strategies, Ithaka Institute. Nikolas Hagemann is employed by Agroscope. Kathleen Draper is the director of Ithaka Institute for Carbon Intelligence.

Author Contributions

- Hans-Peter Schmidt conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Nikolas Hagemann analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Kathleen Draper analyzed the data, authored or reviewed drafts of the paper, approved the final draft.
- Claudia Kammann conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, approved the final draft.

Data Availability

The following information was supplied regarding data availability:

There were no raw data used in this literature review.

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