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E. Royer, A.J.M. Jansman

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Resumé
Deze deskstudie geeft een overzicht van de literatuur over effecten van voeding van varkens op de vorming van methaan in het verteringskanaal en het methaan vormend vermogen van mest. De rol van het microbioom in het darmkanaal, fermentatieprocessen en metabole routes die betrokken zijn bij de methaanvorming in de darmkanaal van varkens worden beschreven. Er is tevens een inventarisatie gemaakt van dier- en voerparameters die gerelateerd zijn voor de hoeveelheid methaan die in het verteringskanaal wordt gevormd. De invloed van voersamenstelling (grondstof- en nutrientsamenstelling en gebruik van additieven) en voederstrategieën die de vorming van enterisch methaan en/of methaanemissie uit varkensmest kunnen verminderen, zijn geëvalueerd.

Abstract
This desk study reviews literature on the effects of the nutrition of pigs on methane formation in the digestive tract and on methane formation capacity of faeces. The role of gut microbiota, fermentation processes and metabolic pathways involved in the methane formation in the hindgut of pigs are presented. An inventory has been made of animal and diet related parameters accounting for the quantity of enteric methane produced. Interventions on diet composition (feed ingredient and nutrient composition and use of specific additives) and feeding strategies that could mitigate enteric methane formation and/or methane emission from pig manure have been evaluated.

This report can be downloaded for free at https://doi.org/10.18174/657546 or at
at www.wur.nl/livestock-research (under Wageningen Livestock Research publications).

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Foreword

Within the Knowledge and Innovation Agenda (KIA) for Agriculture, Water and Food, the programme 'Methane emission reduction in livestock farming' presents as a priority for research and development the necessity for reducing methane formation from rumen and intestinal fermentation and reducing methane and nitrous oxide emissions from animal barns and manure storage. This report presents the results of a literature study on the effects of nutrition of pigs on methane formation in the gastro-intestinal tract and on methane emission from manure.

The present study was funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) [Klimaatenvelop programma 2022-2024; MMIP Emissiereductie methaan veehouderij].

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Eric Royer
Alfons Jansman

Samenvatting

Dit rapport presenteert een overzicht van de wetenschappelijke literatuur over de effecten van voersamenstelling (grondstof- en nutrientsamenstelling en het gebruik van specifieke additieven) en voerstrategieën op de vorming van methaan in het verteringskanaal van varkens en het methaanvormend vermogen uit mest. De studie is uitgevoerd in het kader van het Klimaat Envelop onderzoeksprogramma gefinancierd door het Ministerie van Landbouw, Natuur en Voedselkwaliteit (LNV). Dit programma ondersteunt de ambities van het Nederlandse Klimaat Akkoord als onderdeel van het Nederlandse klimaat beleid om de emissies van methaan en andere broeikasgassen in 2030 en 2050 substantieel te hebben gereduceerd. Een deel van deze ambities moeten worden bereikt via bijdragen uit de veehouderij sector.

De fermentatie van organische stof in de dikke darm van varkens door het aanwezige microbioom en, na uitscheiding van faeces, in mengmest hangt onvermijdelijk samen met de opname van voer en het hieraan gerelateerde verteringsproces in het dier. De vorming en uitscheiding van enterisch methaan betekent een verlies 0.5-3% aan energiewaarde van het voer voor het varken. Methaan wordt gevormd door de activiteit van een complex microbieel ecosysteem in het verteringskanaal van varkens, waarbij verschillende microbiële groepen een synergistische rol spelen. Er bestaan drie metabole routes van methanogenese naast elkaar: de hydrogenotrofe, acetogene en de methylotrofe route. De hydrogenotrofe route, waarbij CO_2 en H_2 als substraten worden gebruikt, is de meest voorkomende en wordt uitgevoerd door de meeste methanogene archaea in de dikke darm. Methanobacteriaceae is de belangrijkste archaea-familie in de dikke darm van varkens, en hydrogenotrofe *Methanobrevibacter* spp. en methylotrofe *Methanosphaera* spp. zijn de dominante soorten in het microbioom in het maagdarmkanaal van varkens. Een groot aantal archaea-soorten die betrokken zijn bij de methanogenese is echter nog niet volledig geïdentificeerd. De kolonisatie van het maagdarmkanaal van dieren door methanogene archaea is grotendeels onbekend, maar diersoort en ras, leeftijd, voedingspatroon en omgevingsomstandigheden spelen een rol. *Methanobrevibacter* spp. zijn efficiënter in CH_4-vorming dan de andere twee genoemde groepen. Concentraties aan acetaat en propionaat, als eindproducten van de fermentatie van organisch materiaal, in de dikke darm van varkens, zijn voorspellers van de vorming van methaan in het maagdarmkanaal, aangezien CH_4 vorming positief gecorreleerd is met de concentratie aan acetaat en de acetaat:propionaat verhouding, en negatief met de propionaat concentratie.

Er is tot op heden slechts beperkt onderzoek gedaan naar het effect van voedingsfactoren op de enterische CH_4-vorming door de archaea populatie in de dikke darm van varkens. In het maag-darmkanaal neemt de vorming van CH_4 toe van het proximale naar het distale deel van de dikke darm en wordt CH_4 voornamelijk via flatulentie uitgescheiden. Voor het *in vivo* meten van enterische CH_4-vorming is het gebruik van respiratiekamers en het meten van methaanconcentraties in de in- en uitgaande lucht de meest gebruikelijke methode, maar voor herkauwers zijn alternatieve benaderingen of methoden ontwikkeld waarbij gebruik wordt gemaakt van sensoren die CH_4 detecteren of NIR-analyses van feces. Deze methoden zouden kunnen worden aangepast voor gebruik in varkens.

De in de literatuur gerapporteerde gemiddelde waarden voor methaanvorming in het verteringskanaal bedragen 0,8, 2,5 en 6-8 g CH_4 per dag voor respectievelijk biggen, vleesvarkens en volwassen zeugen. De enterische vorming van CH_4 bij varkens is echter afhankelijk van de leeftijd en het lichaamsgewicht van het dier, de voeropname en de chemische en nutritionele samenstelling van het dieet, vooral in relatie tot het gehalte aan fermenteerbare vezels. De fractie verteerbare niet-zetmeelpolysachariden (NSP) lijkt de beste indicator voor de hoeveelheid organisch materiaal die door het microbioom in de dikke darm wordt gefermenteerd en kan worden gebruikt als maatstaf voor de enterische CH_4-vorming. Er zijn vergelijkingen opgesteld om de enterische CH_4-vorming door vleesvarkens en volwassen zeugen te schatten op basis van de dagelijkse opname van de hoeveelheid verteerbaar NSP.
Onderzoek heeft aangetoond dat het verminderen van het NSP-gehalte in de voeding de vorming van CH_4 in het darmkanaal vermindert. Het verlagen van het ruw eiwitgehalte in de voeding of het verhogen van het vetgehalte zou de vorming van fermentatiegassen ook kunnen verminderen.

Er is echter weinig meer gedetailleerde informatie beschikbaar over hoe de samenstelling van het voer en de fermentatiecapaciteit van het dier de CH_4-vorming door varkens beïnvloeden.
De literatuur suggereert ook dat sommige specifieke additieven zoals saponinen, tanninen, plantenextracten en essentiële oliën, bestanddelen van algen en specifieke remmers voor methaanvorming (bijvoorbeeld 3-NOP, organische zuren) de methanogenese zouden kunnen verminderen. Er is echter beperkte informatie over dit onderwerp beschikbaar bij niet-herkauwers. Er moet zowel *in vitro* als *in vivo* onderzoek worden uitgevoerd om de werkzaamheid ervan in de praktijk te onderzoeken.

De totale CH_4-emissie uit de varkenshouderij bestaat uit de enterische gevormd methaan door dieren en methaan vrijgekomen uit mest tijdens de stal- en buitenopslag. Afhankelijk van de omstandigheden in de stal en het mest management vertegenwoordigt enterisch methaan 8 tot 20% van de totale CH_4-emissie op varkensbedrijven. Ongecontroleerde vergisting van mest tijdens het verzamelen en opslaan resulteert in de vorming van methaan dat in het milieu wordt uitgestoten, terwijl gecontroleerde anaerobe vergisting van organische stof in mestvergisters resulteert in methaan dat als biogas wordt gebruikt. Het methaanpotentieel van mest wordt beïnvloed door de samenstelling van het voer en met name het organische stof gehalte van de mest. Gebruik van meer circulaire en vezelrijke voeders voor varkens kan leiden tot een hogere CH_4 emissie uit mest. Dergelijke voeders hebben een lagere verteerbaarheid van nutriënten en resulteren in een hogere uitscheiding van fermenteerbare organische stof. De kennis is beperkt over de impact van de samenstelling van het voer op de totale CH_4-vorming en -emissie uit zowel de varkensstal (enterisch methaan en methaan gevormd en geëmitteerd in het hok en in de mestkelder onder een stal) als tijdens de mestopslag en -toepassing buiten de stal. Bovendien ontbreekt het, ondanks interessante resultaten in *in vitro* studies, aan informatie over de effecten van voeradditieven op de CH_4-emissie uit varkensmest. Slechts enkele studies hebben de archaea onderzocht die verantwoordelijk zijn voor de vorming van methaan in de verteringskanaal en in de mest van varkens en hun onderlinge relatie.

Toekomstig onderzoek moet niet alleen zijn gericht op de effecten van voersamenstelling op methaanvorming en -emissie maar ook op andere broeikasgassen en gasvormige emissies die op verterings- of mestniveau ontstaan (b.v. CO_2, NH_3, N_2O, H_2, H_2S). Ten slotte kunnen voermaatregelen om de methaanemissie door varkens terug te dringen niet los worden gezien van andere managementmaatregelen die verband houden met de huisvesting van dieren en de opslag van mest. Onderzoek waarbij gebruik wordt gemaakt van modelleringen geeft aan dat het combineren van maatregelen ruimte zou kunnen creëren voor verdere vermindering van de CH_4-vorming en -emissie in de varkenshouderij.
Er is behoefte aan meer onderzoek om experimenteel en in de praktijk het potentiële vermogen van voersamenstelling en voedingsstrategieën op de emissie van CH_4, andere broeikasgassen en ammoniak te verminderen.

Summary

This report presents a review of the scientific literature on the effects of diet composition (i.e. ingredient and nutrient composition and effects of specific feed additives) and feeding strategy on the enteric formation of methane in pigs and on the methane potential of manure from pigs. The study has been carried out in the framework of the Climate Envelop programme funded by the Dutch Ministry of Agriculture, Nature and Food Quality (LNV). This program supports the ambitions of the Dutch Climate Agreement as part of the Dutch climate policy to reduce the emission of methane and other greenhouse gasses (GHG) in 2030 and 2050. Part of the ambitions should be realized via reductions in the livestock sector.

The fermentation of organic matter in the large intestine by the present microbiome and, after excretion, in the manure, is an important and unavoidable aspect of the intake and digestion of diets by pigs. As methane contains energy, its enteric formation corresponds to a loss of 0.5 to 3% of the amount of digestible energy present in the diet. Methane is formed by specific microorganisms of the archaeal domain. The formation is the result of the activity of a complex microbial ecosystem requiring the synergistic contribution of several microorganisms under anaerobic conditions. Three metabolic pathways of methanogenesis coexist, the hydrogenotrophic, acetogenic and methylotrophic pathway. The hydrogenotrophic pathway, using CO_2 and H_2 as substrates, is the most common pathway and is performed by most of the methanogenic archaea of the colon. Methanobacteriaceae is the main archaea family in the colon of pigs, and hydrogenotrophic *Methanobrevibacter* spp. and methylotrophic *Methanosphaera* spp. are the dominant species in the microbiome in the gastro-intestinal tract (GIT) of pigs. However, a large number of archaea species involved in methanogenesis are not yet fully identified. The acquisition of methanogenic archaea by the host is largely unknown but animal breed, age, dietary and environmental conditions play a role. *Methanobrevibacter* spp. are more efficient in CH_4 formation than the other two mentioned species. Concentrations of acetate and propionate, as end products of fermentation of organic matter in the hindgut of pigs, in digesta in colon are significant predictors of enteric methane formation, as CH_4 is correlated positively with the concentration of acetate and the acetate: propionate ratio, and negatively with the propionate concentration.

At present, only a few studies on dietary factors influencing the enteric CH_4 formation have investigated the archaeal population present in the hindgut of pigs. In the gastro-intestinal tract, CH_4 formation increases from the proximal to the distal part of the large intestine and CH_4 is predominantly released in the air via flatulence. For measuring *in vivo* enteric CH_4 formation, the use of respiration chambers and measurement of methane concentrations in in- and outgoing air is the most common method but alternative approaches or methods using sensors detecting CH_4 or NIR analysis of faeces have been developed for ruminants. They could be adapted for use in pigs.

Average enteric methane formation reported in the literature is 0.8, 2.5 and 6-8 g CH_4 per pig per day for piglets, fattening pigs and adult sows, respectively. The enteric formation of CH_4 in pigs, however, is dependent of the age and body weight of the animal, the feed intake and the chemical and nutritional composition of the diet, specifically in relation to content of soluble fibre. The digestible non-starch polysaccharide fraction (NSP) appears as the best indicator of the amount of organic matter fermented in the colon by the microbiome and can be used as a proxy for enteric CH_4 formation. Equations have been proposed to estimate the enteric CH_4 formation by pigs or adult sows from the daily intake of digestible NSP. Research has shown that decreasing the dietary NSP content can reduce enteric CH_4 formation. Reducing the dietary crude protein content or increasing the fat content could mitigate the enteric gas formation as well. However, little detailed information is available on how the composition of the diet and fermentation capacity of the animal influence the CH_4 formation by pigs. Only limited information is available on the effects of feeding strategies to mitigate CH_4 formation in commercial farming conditions.
The literature suggests that some specific feed additives such as saponins, tannins, plant extracts and essential oils, constituents of algae and methane inhibitors (e.g. 3-NOP, organic acids) could mitigate methanogenesis. However, limited information on this subject is available in non-ruminants. *In vitro* research as well as *in vivo* investigations should be undertaken to explore their efficacy under farming conditions.

Total CH_4 emission from pig houses consists of enteric formation by animals and release from manure during the in-house and outdoor storage. Depending of the animal housing and manure management conditions, enteric methane represents 8 to 20% of the total CH_4 pig farm emission. Uncontrolled fermentation in manure during collection and storage results in formation of methane that is emitted into the environment, while controlled anaerobic fermentation of organic matter in manure in digesters results in methane used as biogas. The methane potential of manure is affected by diet composition. A higher CH_4 release from the manure may result from using circular and fibre-rich diets for pigs. Such diets have a lower nutrient digestibility and result in a higher excretion of fermentable organic matter. However, still little information is available on the impact of the composition of the diet on total CH_4 formation and release from both pig house (enteric methane and methane formed and released in the pen and manure pit underneath a pen) and during manure storage and application outside the barn. Furthermore, in spite of interesting results from *in vitro* studies, information about effects of feed additives on the CH_4 emission from pig manure is lacking. In addition, only a few studies have investigated the community of archaea responsible for methane formation in the GIT and in the manure of pigs and their relationship.

Future research should focus not only on the effects of feed composition on methane formation and emissions but also on other greenhouse gases and gaseous emissions produced at digestive or manure level (i.e. CO_2, NH_3, N_2O, H_2, H_2S). Lastly, dietary mitigation measures to reduce methane emission by pigs cannot be separated from other management measures related to animal housing and manure storage. Research using modelling approaches indicates that combining measures could further reduce CH_4 formation and emission in pig production.
Overall, there is a need for more research to experimentally validate the potential ability of diet composition and feeding strategies to reduce emission of CH_4, other GHG and ammonia in both controlled and field conditions.

1 Introduction

1.1 Background

As part of the global effort against climate change, the Netherlands is committed to make the agriculture and horticulture sector operate in a climate-neutral way by 2050. The European Union has made firm obligations to reduce greenhouse gas (GHG) emissions by at least 40% by 2030 compared to 1990. The Dutch government is implementing policies to achieve a 60% reduction by 2030, and from 80% to 95% by 2050 (Ros and Daniëls, 2017). In addition, the Dutch government as part of the National Methane Strategy (2022) has decided to reduce methane emission in 2030 by 30% compared to 2020. A large share of the reduction should come from the agricultural sector as highest contributor to methane emission in The Netherlands (76% of total).

The Dutch livestock sector need to make a significant contribution to achieve these targets. Ruminants are primarily responsible for CH_4 emission in the Dutch animal production sector, but also pigs contribute substantially to the total emission (Figure 1; van Bruggen et al., 2020).

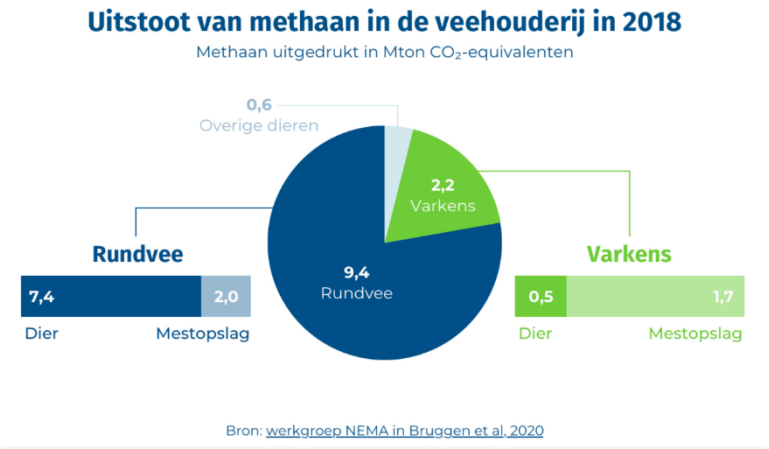
The Knowledge and Innovation Agenda (KIA) for Agriculture, Water and Food[1] has been implemented for 2020 - 2023 with six missions in the field of agriculture, water and food. These missions organize how government, companies, knowledge institutions and citizens work together for the future (https://kia-landbouwwatervoedsel.nl/). The mission B of the Knowledge and Innovation Agenda is dedicated to a 'Climate-neutral agriculture and food production'. It is aimed that by 2050, greenhouse gas emissions have been largely reduced and will be compensated by additional sequestration of carbon dioxide (CO_2) in the soil and in nature. Moreover it states that the sector will no longer use fossil raw materials and will be a supplier of renewable energy. The livestock production sector releases methane (CH_4) and nitrous oxide (N_2O) as major greenhouse gases.

In the atmosphere, methane may retain heat 28 times more than CO_2, calculated over 100 years. However, the lifetime of methane (between 10 and 15 years) is shorter than that of CO_2. Nitrous oxide (N_2O) is released by natural processes in the soil, but can also arise in certain animal housing systems. According to current understanding, nitrous oxide is 298 times more potent than CO_2 in retaining heat in the environment, and therefore considered an important GHG.

The programme B1 'Methane emission reduction in livestock farming' is one of the six Multi-year Mission-Driven Innovation Programmes (MMIP[2]) of the Mission B 'Climate-neutral agriculture and food production'. The aim of this MMIP is to make a maximum contribution to realise the reduction of greenhouse gas emissions from livestock farming (https://kia-landbouwwatervoedsel.nl/wp-content/uploads/B1-Emissiereductie-methaan-veehouderij.pdf). Basically, there are two ways to reduce methane emissions from animals: ensuring that animals emit less methane and reducing emissions from animal manure.

The above mentioned research and innovation MMIP presents two main priorities for which research, development and demonstration phases have been or will be planned:
- Reducing methane formation from rumen and intestinal fermentation.
- Reducing methane and nitrous oxide emissions from animal stables and manure storage.

[1] KIA: Kennis- En Innovatieagenda Landbouw, Water, Voedsel
[2] MMIP: Meerjarige Missiegedreven Innovatieprogramma's



Reproduced from: van Bruggen et al., 2020.

Figure 1 *Methane emissions from Dutch livestock farming in 2018.*

Methane is produced when organic matter is broken down by microorganisms under anaerobic conditions. In practice, this means that methane is mostly produced during rumen and intestinal fermentation in ruminants and in manure storage of cattle and pigs by a specialised group of anaerobic microorganisms, the methanogenic archaea. However, some non-ruminant species, such as pigs also, produce significant amounts of CH_4 in the digestive tract. Recent research did not support a traditional dichotomy of CH_4 formation intensity, when corrected for body weight or feed intake capacity, between ruminant and non-ruminant animal species (Clauss et al., 2020). Several rodent hindgut fermenters may emit CH_4 of a magnitude as high as ruminants expressed at similar size, intake level, or gut capacity. In most animal species, absolute CH_4 formation (l/day) increases linearly with feed and dry matter (DM) intake. However, it is not totally understood which animal and diet related factors determine total enteric CH_4 formation.

For pig farms, besides housing measures and modification of manure management on the farm, targeted approaches through nutrition and feed strategy could influence enteric methane formation and methane forming capacity of pig manure.

The enteric formation of methane in pigs is variable and depends mainly on age of the animal and the presence of fermentable substrate in the diet. Formation levels are around 0.8, 2.5 and 6-8 g CH_4 per animal per day for piglets, fattening pigs and sows, respectively (Phillippe and Nicks, 2015). This corresponds to a loss of 0.5 to 3% of the digestible energy of the diet lost as CH_4 (Jørgensen et al., 2011).

The methane forming capacity of the manure after excretion is also significantly determined by the substrates available for fermentation in the faeces and manure originating from the diet and the host (endogenous secretions released in the gut during the digestive process in the GIT) and by the microbial composition of the manure. The latter could be influenced by the composition of the faecal microbiome and the environmental microbiome that is in close contact with the manure during storage and during further processing and application as fertilizer or input for biogas production.

1.2 Aim of the study

The present report aims to review existing knowledge on the mechanisms of enteric CH_4 formation and on the influence of diet composition and feeding strategies on methane formation by pigs, also considering methane emission capacity from manure.

The report aims to answer some specific questions related to the enteric formation of CH_4:

- ✓ Which microorganisms are methanogenic and what is the role of the microbiota in the formation of methane in the digestive tract?
- ✓ What is the relationship and dependence of fermenting bacteria and archaea (i.e. methanogens) for the formation of hydrogen (H_2), CH_4 and NH_3 gases in the digestive tract.
- ✓ In which part of the intestine is methane formed, and what volumes are produced?

- ✓ What are the substrates or components of the diet that are used for CH_4 formation in the GIT?
- ✓ What ingredients favour CH_4 formation?
- ✓ What are the interactions among age or physiological stage and diet related factors influencing CH_4 formation?
- ✓ How are enteric CH_4 and NH_3 formation linked? Is it possible to reduce simultaneously enteric CH_4 and NH_3 formation?
- ✓ What are the consequences for CH_4 formation of:
    - o the circularity of diets for pigs? Do circular or low-impact ingredients in diet increase or decrease methane formation?
    - o the breeding for more nutrient efficient pigs? Do efficient animals produce less enteric methane?

About CH_4 emissions from manure:

- ✓ Is the methanogenic population of the manure linked to the microbiome population in the gut of pigs?
- ✓ What are the substrates or components of the diet that are used for methane formation in manure?
- ✓ What dietary interventions are available to mitigate CH_4 formation in the gut and in manure?

An inventory has been made on available knowledge in the literature on methanogenic microbial population in the pig gut and on mechanisms related to CH_4 formation. Studies on the contribution of feed ingredients and complete feeds (ingredient composition, nutrient composition, dietary inclusion of specific ingredients or additives) and feeding strategies on enteric methane formation in pigs (piglets, fattening pigs and adult sows) were reviewed. Information on the influence of feeding on the methanogenic intestinal microbiome and CH_4 forming capacity of manure was also reviewed. Attention is also paid to synergistic and possible conflicting effects of diet composition and feeding strategy on enteric CH_4 formation and CH_4 emission from manure on the one hand, and emission of ammonia from pig manure on the other hand.

The results of the literature review and the possibilities to further reduce methane (and ammonia) formation through pig feed are of interest for the entire pig production chain (breeding sector, producers of agricultural raw materials, animal feed sector, and primary pig producers), as well as the Dutch government and society. This study will be a starting point for further experimental studies on the effects of diet composition and feeding strategies on the reduction of formation of enteric methane in pigs and on the reduction of methane and ammonia emission from manure of pigs.

2 Role of the microbiome in enteric CH_4 formation in pigs

In this chapter, the mechanisms of the methane formation in the gut of non-ruminants are described. Methane (CH_4) formation is a ubiquitous, apparently unavoidable consequence of fermentative digestion by the microbiome in the gastro-intestinal tract (GIT) of mammalians. The methanogenesis is a fermentation process that uses CO_2 and H_2 to produce CH_4 and H_2O (water). For pigs or other non-ruminants this gas formation appears in the large intestine as an end product of anaerobic respiration by a specialised group of microorganisms, the methanogens.

2.1 Digestion mechanisms in pigs

2.1.1 Description of essential digestion and fermentation processes

Digestion is the break-down of feed occurring along the digestive tract (Figure 2). Mechanisms of digestion by pigs have been described by Laplace et al. (1986), Rowan et al. (1997) and Lærke and Hedemann (2012). The digestion of feed begins in the mouth where feed is masticated in small fragments and mixed with saliva. Saliva contains salivary amylase, an enzyme which starts the digestion of starch in the feed, and hydrogen carbonate, which provides the ideal alkaline conditions of pH for amylase activity. As the time spent in the oral cavity is short, enzymatic digestion is not of quantitative importance here.

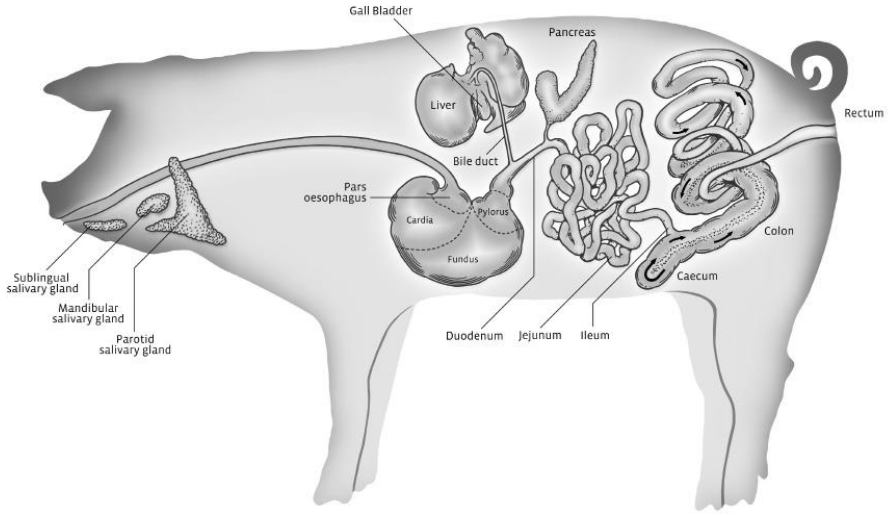


Illustration by Mads Salicath, reproduced from: Lærke and Hedemann, 2012.

Figure 2 *The porcine digestive system.*

Feed is swallowed, then moves down in the form of a round wet mass called a bolus, trough the oesophagus into the stomach. Once in the stomach, gastric juice that mainly contains hydrochloric acid and pepsin starts protein digestion. Hydrochloric acid also provides acidic pH favourable for pepsin activity. Mucus and mineral bicarbonate are also secreted by the stomach to provide a slimy layer on the stomach wall that protects against the damaging effects of hydrochloric acid. At the same time, peristalsis, muscular contractions of the gut that move along the stomach and intestinal tissue, allows the mass of digesta to further mix with the digestive enzymes.

When the pyloric sphincter of stomach opens, the partially digested feed, called chyme, moves to the small intestine where most of the nutrients are hydrolysed and subsequently absorbed by the intestinal mucosa.

In the duodenum, the first section of small intestine, the secretions from the liver and pancreas are added and facilitate digestion. Secretions from the liver are stored in the gall bladder and pass into the intestine through the bile duct. These bile secretions aid in the digestion of fats. Secretions from the pancreas pass through the pancreatic duct and contain enzymes that are central for the digestion of fats, carbohydrates, and proteins. The cells along the wall of the small intestine, or mucosa also produce enzymes that aid digestion and the villi, i.e. finger-like projections of the mucosa, increase the absorptive area of the intestine. After that, a large part of the nutrients are absorbed into the blood in the second and third parts of the small intestine, i.e. the jejunum and the ileum.

Undigested constituents of dietary origin and endogenous secretions pass through the ileo-caecal valve in to large intestine (i.e. caecum and colon), the last major part of the digestive tract. The large intestine is shorter than the small intestine but larger in diameter, and is slightly acidic. Its main function is the reabsorption of water and electrolyte minerals (sodium, chloride, magnesium and potassium) into the blood. Some vitamins, such as biotin and vitamin K, produced by bacteria in the colon, are also absorbed into the blood in the colon. The large intestine is a reservoir for undigested feed residues and endogenous components (e.g. sloughed cells, mucins, enzymes, and bile components) that are substrates for microbial fermentation.

Microbial fermentation occurs in all segments of the gastrointestinal tract. In the first segments, the transit is rapid with little or no accumulation of digesta at any point which is not favourable for bacterial growth, although some lactic acid is produced in the stomach and the distal small intestine, reflecting limited microbial activity in these segments.

The caecum and the colon are characterised by substantial anaerobic fermentation related to low oxygen concentration, high moisture content, long transit time and neutral pH, which are factors favouring bacterial fermentation. The microbial ecosystem contains several hundreds of species of anaerobic bacteria, each species occupying a particular niche with numerous interrelationships. The microbial density reaches approximately 10^{11}-10^{12} viable counts per gram fresh material whereas upper digestive segments have relatively lower numbers of bacteria, with 10^{1}–10^{3} cfu[3]/mL in the lumen of the stomach and duodenum, and 10^{4}–10^{7} cfu/mL in the lumen of the jejunum and ileum (Ewing and Cole, 1994; O'Hara and Shanahan, 2006). A higher diversity and richness of the microbiota in the caecum and the colon is also shown by studies using sequencing of the 16S rRNA gene (Holman et al., 2017). In this segment of the GIT, non-starch polysaccharides (NSP) or dietary fibre are degraded by the microbiome at variable extents, depending of the nature of the carbohydrate polymers present and the degree of lignification (Bach-Knudsen and Lærke, 2012).

The end products of fermentation in the large intestine are short-chain fatty acids (SCFA), fermentation gases and microbial biomass, whereas a certain fraction of NSP in lignified cell walls will not be degraded and is passed to the faeces (Bach Knudsen and Lærke, 2012). Jensen and Jørgensen (1994) measured that as for other monogastric animals and humans, five gases, N_2, 0_2, $C0_2$, H_2, and CH_4, appear to constitute more than 99% of the total amount of gas formed in the GIT of pigs. The flux of SCFA across the colonic epithelia cell depends on the age of the animal and composition of the diet. As reviewed by Lærke and Hedemann (2012), SCFA are the far most important fuel (60-70% of the energy supply) for the colonic mucosa, and butyrate is particularly required to support growth and abundance of epithelial cells. SCFA absorbed from the colon are taken up in blood in the mesenteric veins and transported via the portal vein to the liver. For growing pigs, SCFA can provide 5 to 12% of the energy supply (Laplace et al., 1986).

2.1.2 Classification and digestion of carbohydrates and fibres

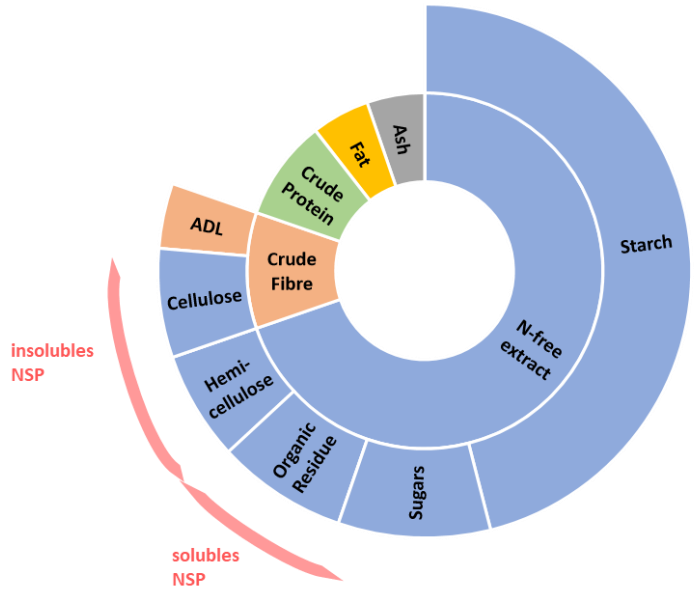
The digestibility of energy and nutrients in feed is largely determined by the chemical composition of the feed (Bach Knudsen and Lærke, 2012). The conventional system to estimate the digestibility of nutrients is the 'Weende' system of analysis or proximate analysis. This system is presented in the inner circle of Figure 3 and consists of the following analyses: dry matter, ash, crude fat, crude protein (i.e. nitrogen ×6.25) and crude fibre. Crude fibre is determined as the ash-corrected insoluble residue after reflux of the fat-extracted residue with 1.25% sulphuric acid and 1.25% sodium hydroxide, which degrade most of the carbohydrates.

[3] Cfu: colony forming units

The calculated residual fraction containing most of the carbohydrates is called the nitrogen-free extract (N-free extract). NFE is calculated as the amount of dry matter not accounted for by the sum of ash, protein, fat and crude fibre (Figure 3).

However, the carbohydrates are not very well defined in the Weende system (Bach Knudsen and Lærke, 2012). Particularly, crude fibre accounts for most of the cellulose and a variable proportion of the lignin (i.e. acid-detergent lignin). Furthermore, the N-free extract comprises a heterogeneous mix of remaining carbohydrates presented in outer circle of Figure 3. In feed ingredients as cereals, the major part of the N-free extract consists of starch and sugars. For other fibre-rich feed ingredients such as sugar beet pulp and potato pulp, the N-free extract mainly contains complex fibrous carbohydrates.

Carbohydrates are very diverse molecules that chemically can be classified according to their molecular size (or degree of polymerization, DP) as sugars (DP, 1-2), oligosaccharides (DP, 3-9) and polysaccharides (DP, ≥10) with the latter consisting of starch and non-starch polysaccharides (NSP) and glycosidic bonds (reviewed by Bach Knudsen and Lærke, 2012). Based on the chemical classification (Figure 3), it is possible to group the carbohydrates nutritionally: digestible carbohydrates represent the carbohydrates that can be digested by digestive enzymes of the animal and resulting nutrients can be absorbed in the small intestine (monosaccharides, disaccharides and most types of starch) while non-digestible carbohydrates are the carbohydrates that cannot be degraded by the endogenous enzymes, but potentially can be degraded by microbial fermentation. The non-digestible carbohydrates fraction comprises most oligosaccharides, enzyme-resistant starch and NSP. The NSP fraction is divided in a insoluble- and soluble- NSP fraction.



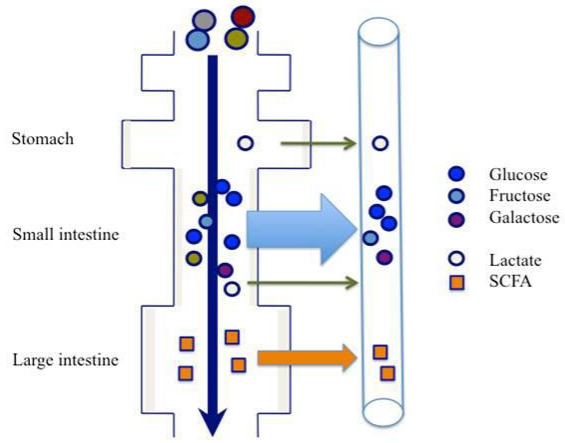
ADL: acid detergent lignin; N-free extract: Nitrogen free extract; NSP: non-starch polysaccharides. All constituents of the carbohydrate fraction are in blue colour.

Figure 3 *Classification of the chemical and nutritional components of a pig diet.*

Intestinal digesta reaching the large intestine contain only small quantities of sugars, oligosaccharides and starch because of their rapid and extensive absorption in the small intestine (Figure 4). Conversely, for cellulose in feed ingredients of plant origin, the relative share of digestion is high in the large intestine. It is estimated that 100% of the cellulose and 60 to 80% of the hemicelluloses of plant derived ingredients are actually digested by pigs by fermentation in the large intestine (reviewed by Laplace et al., 1986). However, the apparent extent of digestion varies according to botanical origin of ingredients and decreases when the level of these cell wall constituents in the feed increases. Fermentation of complex carbohydrates in the hindgut mainly leads to the formation of SCFA and gases including CO_2[4], H_2 and CH_4. An increase in the amount of plant cell wall material (fibre) in the diet increases the amount of substrate for fermentation and the concentrations of SCFA in the colon and the proportion of acetate compared with butyrate and propionate in the cecum.

[4] The IPCC guidelines underline that enteric CO_2 production by domestic animals can be regarded as neutral for environment as the CO_2 expired by livestock in the atmosphere is used for photosynthesis by plants that are fed to animals (Dong et al., 2006).

The digestibility of nutrients in pigs is normally expressed as a digestibility coefficient (%). For most nutrients, including fibre and NSP, apparent faecal digestibility coefficients (total tract digestibility) are used, reflecting the digestibility of nutrients over the entire digestive tract including the small and large intestine. The term "apparent" relates to the point that digesta and faeces also contain so called endogenous constituents, originating from the host and secreted or lost in the digestive tract during digesta passage (e.g. digestive enzymes, mucus and sloughed epithelial cells). Digestibility of protein and amino acids are mostly determined at ileal level and expressed as ileal digestibility coefficients.



Reproduced from: Bach Knudsen and Lærke, 2012.

Figure 4 *Absorption of nutrients derived of the digestion of carbohydrates in the digestive system of pigs.*

2.2 Microbiota involved in methanogenesis in pigs

Methane is produced in the hypoxic conditions of wetlands and in the digestive tract of animals and humans by specific microorganisms called methanogens. Methane is the end-product of their anaerobic respiration. The formation of methane appears to be nearly inevitable in the digestive tract of animals. It is concluded by Clauss et al. (2020) that all mammals harbour some methanogens, and produce some CH_4.

All methanogens are strictly anaerobic archaea belonging to the Euryarchaeota phylum. They are obligate methane producers and obtain all or most of their energy from methanogenesis (reviews of Hedderich and Whitman, 2013; de la Fuente et al., 2019; Misiukiewicz et al., 2021).

Table 1 *Classification of genera of methanogens detected in humans and domestic non-ruminants.*

Class	Order	Family	Genus
Methanobacteria (Methanomamada group)	**Methanobacteriales**	Methanobacteriaceae	*Methanobacterium*
			Methanobrevibacter
			Methanosphaera
			Methanothermobacter
Methanomicrobia (Stenosarchea group)	**Methanomicrobiales**	Methanocorpusculaceae	*Methanocorpusculum*
		Methanomicrobiaceae	*Methanomicrobium*
			Methanoculleus
			Methanogenium
	Methanosarcinales	Methanosarcinaceae	*Methanosarcina*
			Methanimicrococcus
	(Methanotrichales)	Methanosaetaceae (syn. Methanotrichaceae)	*Methanosaeta (syn. Methanothrix)*
Thermoplasmata	**Methanomassiliicoccales**	Methanomassiliicoccaceae	*Methanomassiliicoccus*

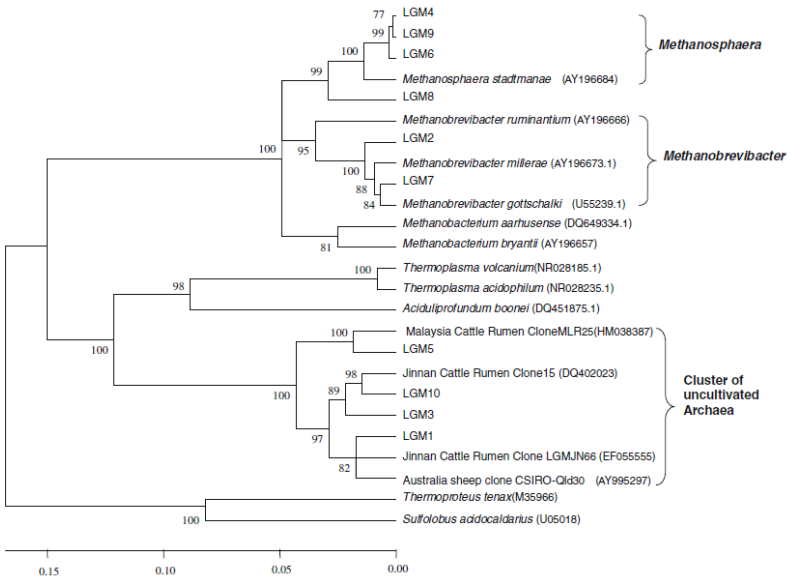
Syn.: synonym. (adapted from Liu and Whitman, 2008; NCBI, 2023).

A recent study of the gut archaeome indicated the presence of archaea in 175 animal species in the animal kingdom ranging from invertebrates to mammals (Thomas et al., 2022). The vast majority (94%) of the reads of marker genes (i.e. 16S rRNA) were affiliated to only five methanogenic genera or order: *Methanobrevibacter*, *Methanosphaera* (Methanobacteriales), *Methanomethylophilaceae* (Methanomassiliicoccales), *Methanocorpusculum* (Methanomicrobiales), *Methanimicrococcus* (Methanosarcinales), and one non-methanogenic (i.e. ammonia-oxidizer) lineage: *Nitrososphaeraceae* (Nitrososphaerales/Thaumarchaeota). These lineages can be qualified as the dominant gut archaea (Thomas et al., 2022).

For non-ruminants, the classification of the archea involved in methanogenesis is still uncompleted, as it has not been widely studied because of the practical constraints for isolating and cultivating species. However, the studies reviewed by Misiukiewicz et al. (2021) have revealed that most methanogens commonly inhabit the digestive tract of several nonruminant species (Appendix 1). Misiukiewicz et al. (2021) underlined that the composition and the density of methanogens in the GIT of monogastric animals vary not only among animal species but is also influenced by location of the GIT, the breed and age of the host and the diet composition. In the digestive tract of chickens, Qu et al. (2008) also indicated the presence of five classes of methanogenic archaea: Methanobacteria, Methanomicrobia, Thermoplasmata, Methanococci, and Methanopyri, but did not investigate these further. A classification of the main genera of methanogens detected in nonruminants is shown in Table 1.

In pigs, phylogenetic analysis of archaeal diversity in colonic digesta have shown the presence of archaea closely related to *Methanobrevibacter* spp., *Methanosphaera* spp., Methanomassiliicoccales and Methanomicrobiales (Luo et al., 2013; Luo et al., 2017; Mi et al., 2019). According to Mi et al. (2019), the Methanobacteriaceae family is the dominant methanogen in colonic digesta of finishing pigs, accounting for approximately 71% of the identified methanogens. In the study of Mi et al. (2019), the search from the colonic digesta of operational taxonomic units (OTUs) of mcrA gene sequences used as biomarker of methanogenesis showed that the most abundant archaea were closely related to *Methanobrevibacter* spp. accounting for 57%, *Methanosphaera* spp. accounting for 14%, Methanomassiliicoccales accounting for 15% and Methanomicrobiales with the lowest occurrence of all identified taxa.

At species level, *Methanobrevibacter* spp. and *Methanosphaera* spp. have been detected in pig faeces (Mao et al., 2011; Luo et al., 2012; Su et al., 2014; Federici et al., 2015). A methanogenic archaeal 16S rRNA gene clone library of pig faeces has been established by Mao et al. (2011) wherein the clones are mainly separated into three clusters: *Methanobrevibacter* spp., *Methanosphaera* spp., and a group of uncultivated archaea (Figure 5).



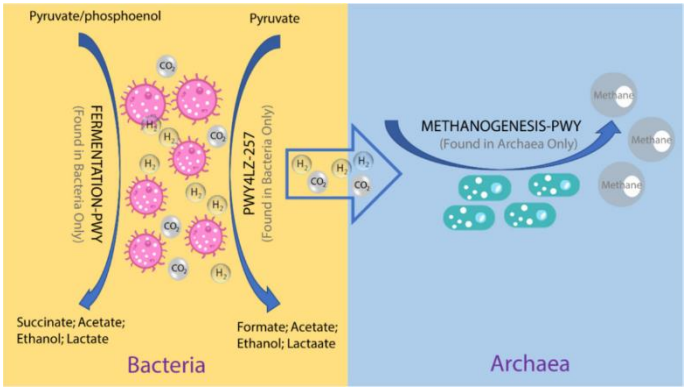
Reproduced from: Mao et al., 2011.

Figure 5 *Phylogenetic relationships of archaeal clones derived from 16S rRNA gene evolutionary distances in pigs.*

In most studies, the presence of some uncultivated and unknown archaea with great similarities to known species but not assigned to a strict taxonomic unit, is revealed, according to Misiukiewicz et al. (2021). In agreement, Mao et al. (2011) estimated that whereas *Methanobrevibacter* spp. were the most abundant of archaea identified in pig faeces, constituting 46% of clones, unidentified archaea species made up 55% of clones. The detection of uncultivated and unknown archaea with 77–80% similarity to known methanogens may indicate the presence of novel undiscovered methanogen species in pigs. Such unidentified euryarchaeotic sequences have been noted in swine (Mao et al., 2011; Luo et al., 2012, 2017; Mi et al., 2019), but also for rabbits (Kušar and Avguštin, 2010) and poultry (Saengkerdsub et al., 2007).

2.3 Enteric methane formation

The methanogenesis pathway is complex as highlighted by de la Fuente et al. (2019), and requires specific coenzymes and membrane-bound enzyme complexes. Methanogens have an extreme genetic diversity, but they can utilize only a limited number of substrates: carbon dioxide (CO_2), acetate and compounds containing methyl groups (Liu and Whitman, 2008; Hedderich and Whitman, 2013). Consequently, de la Fuente et al. (2019) pointed that most organic compounds such as carbohydrates, volatile fatty acids (VFA) and alcohols are not direct substrates for methanogens and have to be fermented first by syntrophic bacteria, protozoa or fungi to acetate, formate, H_2 and CO_2, before their use by methanogens[5]. This is consistent with the metagenomic study of Deng et al. (2021) showing that in pigs the pathways related to H_2 consumption were only observed in archaeal microbiota, while the pathways participating in H_2 formation were only detected in bacterial communities. As part of an organised microbial ecosystem, these bacteria also depend on the association with methanogens to maintain favourable low concentrations of H_2 (Liu and Whitman, 2008). Therefore, in methanogenic environments, most of the available energy for microbial growth is utilized by the non-methanogenic organisms (Liu and Whitman, 2008). The latter authors qualified the fact that methanogens cannot directly degrade and utilize "complex organic matter" and depend on other organisms as a "*physiological mystery*".



Reproduced from: Deng et al., 2021.

Figure 6 *Synergetic collaboration among bacteria and archaea.*

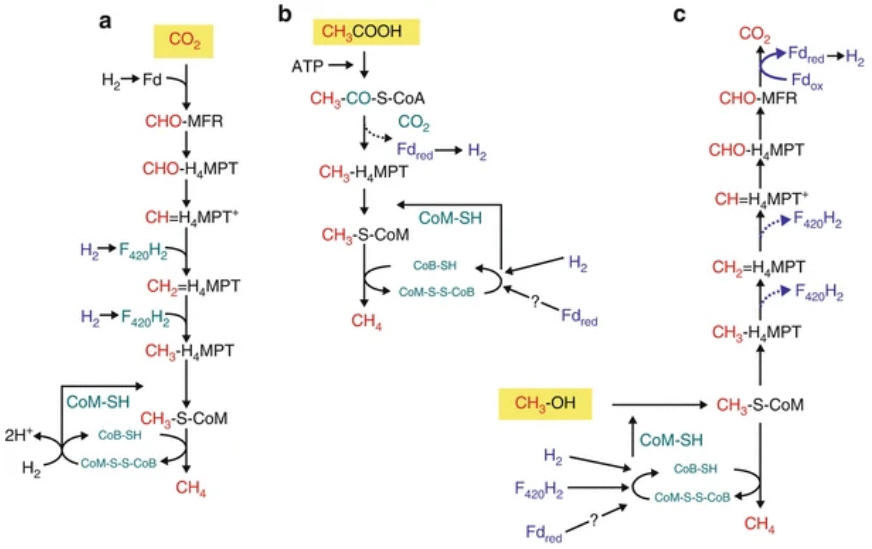
Considering the ecology and evolution of the archaea, these organisms are well adapted to energetic stress. Archaea have the capacity to conserve small amounts of metabolically useful energy during catabolism (i.e. only a fraction of an ATP for each methane molecule produced) and have low-permeability lipid-membranes in order to survive in anaerobic, energy-stressed environments as indicated by Valentine (2007). In terms of cellular bioenergetics, adaptation to chronic energy stress is hypothesized to be the crucial factor that distinguishes the archaea from bacteria.

2.3.1 Metabolic pathways of the methane formation

Three methanogenesis pathways correspond to the metabolic transformation of different substrates for the formation of methane. Methanogenic species are specialised and generally use only one metabolic pathway as shown in Table 2.

[5] See relation of biochemical pathways of H_2, H_2S, CH_4, and CO_2 formation from microbial fermentation in Appendix 2.

For the **hydrogenotrophic pathway** (Figure 7, a), typical of orders Methanobacteriales and Methanomicrobiales, **CO_2** is used by methanogens as the carbon source and electron acceptor while **H_2** is used as the major electron donor (Misiukiewicz et al., 2021). Formate can also provide an electron by the activity of the formate dehydrogenase. In this pathway, CO_2 is reduced to methane through formyl, methylene and methyl, forming C-1 fragment in which methyl-coenzyme M reductase catalyses the last step of this metabolic route (Hedderich and Whitman, 2013). Two methanogen species can also utilise carbon monoxide (CO) as reductant for methanogenesis from CO_2, by using CO dehydrogenase. However, microbial growth with CO is slow and the doubling time is more than 200 h for *Methanothermobacter thermoautoprophicus* and 65 h for *Methanosarcina barkeri* (Liu and Whitman 2008). In contrast, *Methanosarcina acetivorans* utilize CO for growth but by an entirely distinct pathway (Rother and Metcalf, 2004). In addition, some hydrogenotrophic methanogens can also oxidise alcohols (i.e. propanol, butanol, cyclopentanol and ethanol) as the electron donors (Hedderich and Whitman, 2013).



The methyl-coenzyme M (CH_3-S-CoM) is a central intermediate in all three pathways. It is converted to methane and the heterodisulfide of coenzyme M and coenzyme B (CoM-S-S-CoB). CoM-S-S-CoB thus generated functions as the terminal electron acceptor of different respiratory chains. H_2 and reduced coenzyme F_{420} ($F_{420}H_2$) have been identified as electron donors for the reduction of CoM-S-S-CoB. Abbreviations: CHO-FMR, N-formylmethanofuran; CHO-H_4MPT, N ^{5}-formyltetrahydromethanopterin; CH=H4MPT$^+$, N ^{5},N ^{10}-methenyl-tetrahydromethanopterin; CH_2=H_4MPT, N^5,N^{10}-methylene-tetrahydromethanopterin; and CH_3-H_4MPT, N^5-methyl-tetrahydromethanopterin. Reproduced from: Hedderich and Whitman, 2013.

Figure 7 *Methanogenesis pathways from H2 and CO2 (a), acetate (b), and methanol (c).*

A second type of substrate is **acetate** (Figure 7, b). In this reaction, the methyl (C-2) carbon of acetate is reduced to methane using electrons obtained from the oxidation of the carboxyl (C-1) carbon of acetate. The reaction is defined as **aceticlastic pathway** because it results in the splitting of acetate into methane and CO_2. In this reaction, the methyl group enters the C_1 pathway at the level of methyl-H_4MPT. Liu and Whitman (2008) underlined that only two genera: *Methanosaeta* and *Methanosarcina* are known to use acetate for methanogenesis. *Methanosaeta* is a specialist that uses only acetate, including at concentrations as low as 5-20 µM. *Methanosarcina* appears to be a relative generalist with a high growth rate, using also methanol, methylamine or H_2 but with low affinity for acetate, requiring a minimum acetate concentration of about 1 mM (Jetten et al., 1992). In anaerobic digestors used for the degradation of organic wastes, acetate accounts for two thirds of the methane formation (Liu and Whitman, 2008). Interestingly, it appears that only one aceticlastic methanogen group, *Methanosaeta* or *Methanosarcina*, dominates the methanogenesis of anaerobic digestors, depending of the acetate concentration, type of substrate waste used and its feeding rate (Leclerc et al., 2004). On the other hand, methanogenesis from acetate in monogastrics is generally limited, according to Miller and Wolin (1986), because the relatively short retention time of digesta in the GIT does not allow the slow growth of methanogens on acetate. Furthermore, it is important to note that the competition between methanogenesis and acetate formation (or acetogenesis) for H_2 capture may occur in the pig hindgut affecting CH_4 formation (De Graeve et al., 1994) in spite that acetate formation is thermodynamically less favourable than methanogenesis.

In the **methylotrophic pathway** (Figure 7, c), substrates for methane synthesis are the C_1 compounds containing a **methyl group** bound to O, N or S such as methanol, methylamines, methylsufides and others (Figure 4, c).

Consequently, the six amino acids that contain methyl groups (alanine, leucine, valine, isoleucine, threonine and methionine) could theoretically be used as co-substrates for this pathway but this is not reported in biochemistry reviews on methanogenesis. However, protein and amino acid digestibility in the small intestine is rather high, leaving less amino acids available for fermentation in the hindgut. The methyl groups are transferred to a cognate corrinoid binding protein (MtxC) and, subsequently, enter into the methanogenesis C_1 pathway at the level of the methyl-coenzyme M, to be further reduced to methane (Ferguson et al., 2000). Activation and transfer of the methyl group requires a substrate-specific methyltransferase. Methylotrophic methanogens are limited to the order Methanosarcinales, except for *Methanosphaera* spp.(order Methanobacteriales) and some Methanomassiliicoccales. In this pathway, three methyl groups are reduced to methane for every molecule of CO_2 formed (Hedderich and Whitman, 2013). In the presence of both a methyl group donor and H_2, the methyl oxidation is inhibited and the methyl groups are completely reduced to CH_4. Some exceptions are *Methanomicrococcus* spp. and *Methanosphaera* spp. that are obligated methylotrophic and are specialised in reducing methyl groups only when H_2 is also present.

Table 2 *Main archaea groups and methanogenesis pathways in non-ruminant animals.*

Family	Genus	Methanogenesis pathway	Major substrates used
Methanobacteriaceae	*Methanobacterium*	Hydrogenotrophic	CO_2, H_2, formate
	Methanobrevibacter	Hydrogenotrophic	CO_2, H_2, formate
	Methanosphaera	Methylotrophic	H_2, methanol
	Methanothermobacter	Hydrogenotrophic	CO_2, H_2, formate
Methanocorpusculaceae	*Methanocorpusculum*	Hydrogenotrophic	CO_2, H_2, formate
Methanomicrobiaceae	*Methanomicrobium*	Hydrogenotrophic	CO_2, H_2, formate
	Methanoculleus	Hydrogenotrophic	CO_2, H_2, formate
	Methanogenium	Hydrogenotrophic	CO_2, H_2, formate
Methanosarcinaceae	*Methanosarcina*	Methylotrophic, aceticlastic	(H_2), methylamine, acetate
	Methanimicrococcus	Methylotrophic	H_2, methanol, methylamine
Methanosaetacea	*Methanosaeta*	Aceticlastic	Acetate
Methanomassiliicoccaceae	*Methanomassiliicoccus*	Methylotrophic	H_2, methanol, methylamine

Adapted from Liu and Whitman, 2008. Parentheses indicates utilized by some, but not all species or strains.

Of these methanogenesis patterns, the hydrogenotrophic pathway is the most common and is performed by the majority of the methanogens inhabiting the animal GIT according to Misiukiewicz et al. (2021). To a second rank, the methylotrophic pathway is activated by *Methanosphaera* spp. However, whereas the populations of total methanogens and methanobacteriales are usually stable among the different parts of the large intestine (Mi et al., 2019), any higher or lower presence of certain methanogens could lead to an evolution in the occurrence of a particular metabolic pathway[6]. *Methanobrevibacter* and *Methanosphaera* are the two dominant H_2-consuming genera that are usually found in the GIT of animals or humans (Gaci et al., 2014; Liu et al., 2018; Mi et al., 2019). Furthermore, whereas *Methanobrevibacter* produces one mole of methane per mole of CO_2, Methanosphaera requires four moles of methanol to produce three moles of CH_4 (Liu et al., 2018). This explains that Methanosphaera spp. produce smaller amounts of CH_4 than *Methanobrevibacter* spp. Consequently, an increase in the abundance of *Methanobrevibacter* spp. and a decrease in *Methanosphaera* spp., along with a decrease in the diversity of methanogens, promoted CH_4 formation in the study of Liu et al. (2018). Lastly, few *Methanosarcina* spp. and *Methanosaeta* spp., able to use acetate as substrate, are identified in the GIT of animals (Murru et al., 2018; Mi et al., 2019; Thomas et al., 2022).

[6] An original pathway of metabolic hydrogen utilisation such as acetogenesis competes with methanogenesis, whereas this pathway is practically negligible in the rumen (Review of Vermorel et al., 2008).

2.3.2 Interactions with the bacterial fermentations

Methanogenesis illustrates an interesting biochemical interdependency between microbes, as underlined by Pimentel et al. (2013). Methane formation in animals is mainly dependent on the presence of H_2 for the reduction of CO_2 or methyl compounds, i.e. hydrogenotrophic and methylotrophic pathways. With CO_2, H_2 is the most predominant gas produced by colonic bacteria and is produced solely through bacterial fermentation (Naito et al., 2018). In the gut, the main bacterial genera involved in H_2 formation through anaerobic oxidation of the non–digestible substrates are *Bacteroides*, *Ruminococcus*, and *Roseburia*. *Anaerostipes caccae*, *Clostridium* spp., *Eubacterium rectale*, Enterococcus, and *Victivallis vadensis* also produce H_2 (reviewed by Mutuyemungu et al., 2023). However, the accumulation of H_2 is intoxicating these micro-organisms and slows their metabolism. Partnering with H_2-utilizing microorganisms as methanogens prevents H_2 accumulation, thereby favouring optimal efficiency of the H_2-producing bacteria. Since 4 moles of hydrogen are needed to produce 1 mole of methane, the methanogenic metabolism is very efficient in removing hydrogen. This role is shared with two other types of anaerobic H_2-utilizing sulfate-reducing bacteria (SRB) that produce H_2S (*e.g.*, *Desulfovibrio* spp.), and acetogenic bacteria (*e.g.*, *Ruminococcus* spp). The investigations of Pochart et al. (1992) have shown a competitive interrelation between methanogenic archaea and SRB in the colon of humans. However, it did not lead to a complete mutual exclusion of the two populations, contrary to the hypothesis based on the previous studies of Gibson et al. (1988, 1990).

Furthermore, methanogens are abundant in habitats where electron acceptors such as O_2, NO_3^-, Fe^{3+}, and SO_4^{2-} are limiting (Liu and Whitman, 2008). When electron acceptors other than CO_2 are present, methanogens are outcompeted by the bacteria that utilize electrons, such as sulfate-reducing bacteria (SRB), denitrifying bacteria, and iron-reducing bacteria. This phenomenon probably occurs because these compounds (i.e. NO_3^-, Fe^{3+}, and SO_4^{2-}) are better electron acceptors, and their reduction is thermodynamically more favourable than CO_2 reduction to methane. However, as CO_2 is generated in fermentation processes, it is rarely limiting in anaerobic environments.

2.4 Microbiota ecology and evolution of methanogens in the digestive tract of pigs

2.4.1 Development of methanogens in the GIT of pigs

Substantial inter-individual differences exist in colonic methanogens in humans (Miller and Wollin, 1986; Pochart et al., 1992). The abundance of methanogens in human faecal samples varies from undetectable to 10^9 cfu per g of faeces. From the observations for humans reviewed by Mutuyemungu et al. (2023), it could be anticipated that methanogenic activity could also be variable among individual pigs. But, in contrast to this hypothesis, little variation of the CH_4 formation rate among pigs was observed by Robinson et al. (1989) for pigs fed the same corn soybean meal diet. Accordingly, Mi et al. (2019) found a stable pH, total number of methanogens, and Methanobacteriales in the large intestine of individual finishing pigs. The former may be related to the fact that all pigs received a similar diet. Indeed, as most pigs are reared in groups in controlled environments and fed with diets adapted to their nutrient requirements for growth and maintenance, it is realistic to assume that the differences in methanogenesis between individuals are smaller than in other species consuming less standardized diets.

Factors that determine the acquisition of methanogenic archaea in mammalians are mainly unknown. In pigs, the archaeal composition in the GIT is dynamic (Luo et al., 2017) and appears as significantly affected by the age and the breed of pigs. The early methanogenic colonisation in faeces of Meishan and Yorkshire neonatal piglets was dominated by members of the genus *Methanobrevibacter*, represented by *M. smithii*, *M. thaueri*, and *M. millerae*, as shown by Su et al. (2014). It was found in this study that the diversity of the methanogenic community decreased from one to 14 days of age, whereas the total methanogen populations increased. For the first 14 days of life, the abundance of *M. smithii* increased significantly, while the abundances of marker genes (i.e. operational taxonomic units, OTUs) related to *M. thaueri* and *M. millerae* decreased significantly.

Interestingly, this substitution was faster in lean Yorkshire piglets than in fat Meishan piglets. At weaning, Federici et al. (2015) also reported a shift in archaeal composition with *Methanobrevibacter boviskoreani* replacing *M. smithii*.

A probable explanation of the prevalence of some archaea species could be the difference in metabolic activity. Whereas two Methanobacteriales, *Methanobrevibacter smithii* and *Methanosphaera stadtmanae* are found in 50 and 33% of the adult human population, respectively, Gaci et al. (2014) underlined that *M. smithii* is more efficient for methanogenesis and produces the majority of methane.

Environmental parameters may also play a crucial role in gut colonization. This view is supported by the study of Florin et al. (2000) who conducted statistical modelling of data on the composition of exhaled breath samples from human adolescent twin pairs and their families, and using data from experiments studying cohabiting methanogenic and non-methanogenic rats. The results demonstrated that main factors that influence the occurrence of methanogenesis in the hindgut of animals and the colon of humans are shared environmental factors, and not genetic factors. These factors were most strongly operative during the postweaning period of rats. The precise nature of the microbial and host ecological factors that prevent colonization with methanogens were unexplained.

The ecology and growth of archaea is also largely influenced by the availability of energy (Valentine, 2007; Moissl-Eichinger et al., 2018) and consequently by the intake of feed by farm animals. Lastly, abundance, diversity and composition of methanogens in hindgut of pigs may be affected by fibre content, diet type and microbial status of the diet. For humans, the gut colonization by *Methanobrevibacter smithii* of the children in the Netherlands was correlated with the consumption of organic dairy products in which *Methanobrevibacter smithii* were present (Van de Pol et al., 2017). For pigs, a limited number of studies showed that the diversity and activity of methanogens can be influenced by the diet. Level and source of dietary fibre have a major impact on methanogenic community structure and CH_4 formation pathways, as shown for e.g. pea fibre by Luo et al. (2017; Figure 8). In the case of rabbits, decreasing the particle size of fibre source decreased methanogen diversity, and increased the abundance of *Methanobrevibacter* spp. at the expense of *Methanosphaera* spp., which likely resulted in increased CH_4 formation (Liu et al., 2018). In the study reported by Seradj et al. (2018), growing pigs fed high protein diets showed greater abundances of methanogens than those fed low protein diets. However, in the same study, pigs receiving a high amount of dietary fibre (from sugar beet pulp, rapeseed meal and sunflower meal) tended to emit more CH_4, but did not differ in methanogenic archaea concentrations in the hindgut. Interestingly, the study conducted by Cao et al. (2016) showed that gilts fed low fibre diets may have a higher density of 16S ribosomal RNA genes of *Methanobrevibacter* spp. than gilts given rice bran and hulls in both the in vivo and in vitro trials. Lastly, the supplementation of specific polysaccharide (e.g. β-glucan) to the diet of pigs may increase the diversity of intestinal methanogens (Luo et al., 2013).

Similar species	Piglet-C	Piglet-P	Finisher-C	Finisher-P
Methanobrevibacter millerae	70.77	48.42	46.91	28.57
Methanomassiliicoccus luminyensis	0.00	36.32	9.28	20.41
Methanobrevibacter smithii	14.36	0.00	19.07	19.39
Methanobrevibacter gottschalkii	9.23	4.21	0.00	17.35
Methanobrevibacter olleyae	0.51	0.00	4.64	10.71
Methanobrevibacter ruminantium	1.03	10.00	20.10	3.57
Methanobrevibacter boviskoreani	4.10	1.05	0.00	0.00

Inclusion levels of pea fibre in diets were 0% (Piglet-C and Finisher-C), 10% (Piglet-P) or 30% (Finisher-P). The background colour of each cell indicates relative abundance of each phylum with red and green indicating highest and lowest values. Reproduced from: Luo et al., 2017.

Figure 8 *Effects of age and dietary pea fibre content on the abundance of methanogens in the colon of pigs.*

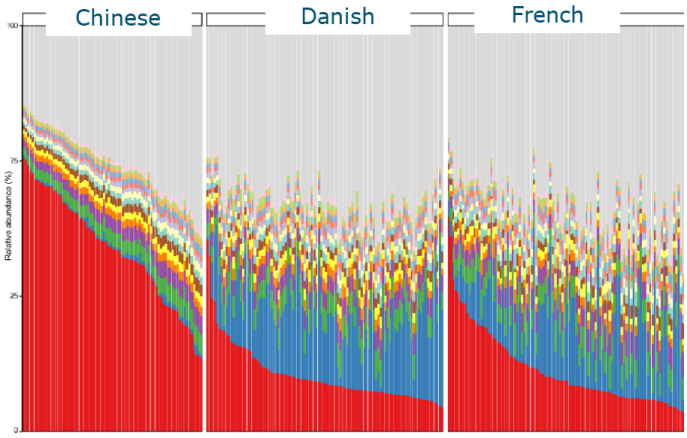
The oxidation-reduction potential (Eh) is an important factor that also influences the microbiome composition. Methanogens are an exclusively anaerobic microbiome that can only grow in low Eh environments. Anaerobes require in general an Eh range from + 100 to - 250 mV. The Eh values in the large intestines of finishing pigs studied by Mi et al. (2019) were from - 297 to - 423 mV which was lower than values in the rumen (- 130 to - 200 mV), indicating that the hindgut of finishing pigs had a stricter anaerobic environment. Overall, the methanogens in pigs require stricter anaerobic conditions and are difficult to isolate and culture compared to those of ruminants. However, the correlation analysis of Mi et al. (2019) between Eh and Methanobacteriales showed that a higher Eh value within the range of the study, improved the growth of Methanobacteriales in the rectum, descending colon, and caecum. The latter authors underlined that whether the high Eh values in the gut of finishing pigs improve the growth of methanogens requires further study.

In addition, VFAs are generated via fermentation in the large intestine and maintain a pH of digesta of generally between 6 and 7. In the study of Mi et al. (2019), however, the pH in the large intestine was between 5 and 7.

2.4.2 Interactions of methanogens with the host metabolism

Most authors agree that methanogens play an important role in energy metabolism and adipose tissue deposition in animals (reviewed by Misiukiewicz et al., 2021). Regarding the energy balance, energy from feed that is converted into CH_4 and released to the atmosphere represents a loss of energy that could have been transformed through other hydrogen metabolic pathways. Indeed, higher participation of acetogenesis in H_2 oxidation, leading to acetate formation, could be a more favourable mechanism to obtain more energy from the diet and reduce the environmental impact of animal production (Misiukiewicz et al., 2018).

Higher abundance of methanogens, along with their higher diversity, have been reported to contribute to a lean phenotype in pigs. In particular, a greater abundance of *Methanosphaera* spp. and early dominance of *Methanobrevibacter smithii* were correlated with a lower retention of body fat in pigs (Luo et al., 2012; Su et al., 2014). In fattening pigs, Luo et al. (2012) showed that the lean Landrace pig had a greater diversity and higher numbers of methanogen genes in faeces than the obese Erhualian pig. These differences may be related to differences in the fatness of these two breeds of pigs. The comparison of OTUs between the two gene libraries showed that Methanobrevibacter-like sequences (97%) were dominant in the faeces of Erhualian pigs, whereas the proportion was 57% for Landrace pigs. Furthermore, Landrace pigs had 10 times more *Methanosphaera*-like methanogens than Erhualian pigs (44 vs 3%). Using faecal samples from three countries, Deng et al. (2021) also found that archaeal communities were less diverse in Chinese pigs than in Danish and French pigs (Figure 9).



Methanobrevibacter (red bars) is overall the most abundant archaeal genus and was the most dominant in Chinese pigs (45%) and French pigs (15%). However, it was ranked at second rank in Danish pigs while *Candidatus methanomethylophilus* (blue bars) was the most predominant in Danish pigs (16%). Reproduced from: Deng et al., 2021.

Figure 9 *Influence of environment and breed on diversity of archaea in 276 pig faecal samples from China, Denmark and France.*

Other studies suggested that the participation of methanogens to the microbiome in the gut can promote energy utilization from the diet and contribute to obesity. *M. smithii* facilitates polysaccharide fermentation by syntrophs, resulting in a higher SCFA formation and enhanced availability of dietary energy (Schink, 1997). Using a gnotobiotic mice model, Samuel and Gordon (2006) have shown that the presence of methanogen *M. smithii* along with *Bacteroides thetaiotaomicron* (a polysaccharide utilizer) resulted in a more efficient digestion of carbohydrates, increased formation and absorption of SCFAs, greater serum acetate levels, increased hepatic *de novo* lipogenesis and host adiposity under conditions of equal feed intake, when compared to mice devoid of methanogens. The study revealed that *M. smithii* directed *B. thetaiotaomicron* to focus on fermentation of dietary fructans to acetate, whereas B*. thetaiotaomicron*-derived formate was used by *M. smithii* for methanogenesis. The *B. thetaiotaomicron – M. smithii* cocolonization of mice gut induced a significant increase in host adiposity compared with mono-association with these species, or *B. thetaiotaomicron – D. piger* (i.e. a SRB) bi-association.

These findings also suggest that the common view that different types of carbohydrates do not differ significantly with respect to their impact on energy retention in the body is related to the variation in fermentative capacity of the intestinal microbiota. Samuel and Gordon (2006) concluded that their findings support a relationship between the energy balance of the host and the bacterial utilization of dietary polysaccharides with involvement of archaea.

The influence of methanogens on health has been mainly investigated for humans. It can be speculated that alterations in the methanogen communities could play a role in obesity of populations exposed to short-chain carbohydrates commonly encountered in the modern diets (Samuel and Gordon, 2006; Basseri et al., 2012; Laverdure et al., 2018). However, Gaci et al. 2014 reviewed that epidemiology studies offered contradictory results about the levels of methanogens in obese persons. In humans, CH_4 formation may also be considered as a potential biomarker to identify diseases in the GIT. High levels of CH_4 have been linked to decreased intestinal transit (Pimentel et al., 2006) and are associated with constipation, especially in human patients suffering from Irritable Bowel Syndrome with constipation (reviewed by Mutuyemungu et al., 2023).

2.5 Conclusion

Enteric methane formation by pigs is the result of the microbial degradation of organic matter in complex anaerobic ecosystems requiring the contribution of several groups of microorganisms linked in a food chain that degrade macromolecules to VFAs and CO_2, H_2 and CH_4 gasses. The main substrates for methane formation are CO_2, H_2, methyl groups and acetate. Organic compounds such as carbohydrates and VFAs as butyrate and propionate, are not direct substrates for methanogens and have to be processed by bacteria, protozoa or fungi, before their utilisation by methanogens. Consequently, bacteria and archaea form together a stable microbial ecosystem.

Among methanogenic archaea that produce CH_4, the Methanobacteriaceae family is the dominant methanogen in colonic digesta of non-ruminants and pigs, accounting for approximately three quarters of the identified methanogens. Two species: *Methanobrevibacter* spp. and *Methanosphaera* spp. dominate the identified flora of pigs but a large number of archaea species involved in methanogenesis are not yet fully identified. Three metabolic pathways of methanogenesis coexist. The hydrogenotrophic pathway from CO_2 and H_2 is the most important but the relative contribution of the pathways can be modified by the conditions in the hindgut. The utilization of H_2 by methanogens prevents H_2 accumulation, which favours optimal fermentation conditions for bacteria. However, the role of methanogenic archaea is shared with sulphate-reducing bacteria and acetogenic bacteria which can be competitors for using H_2 in the colon. Acetate and propionate concentrations in colon can be predictors of methane formation, as CH_4 concentration is correlated positively with the concentration of acetate and the acetate : propionate ratio, and negatively with propionate concentration.

Whereas important variability among individuals for numbers of methanogenic archaea and methane formation may exist for other species as humans, the variability among individual pigs fed the same diet could be less elevated. Factors that play a role in the acquisition of methanogenic archaea are largely unknown. Previous research showed that the abundance and diversity of the methanogen community in pig hindgut is clearly influenced by the host breed and age and by environmental conditions. However, in

humans, the role of genetic factors is discussed. Furthermore, the methanogen composition in the gut may be impacted by factors related to feeding and diet composition such as feed intake, fibre source, protein content and particle size of the fibre fraction. Some studies have established relationships between diet composition and the abundance of methanogens, methanogenic pathway and CH_4 formation in the hindgut. However, other studies observed changes in the CH_4 formation by pigs following a dietary treatment without modification of the abundance of methanogenic archaea.

Energy from the diet that is converted into CH_4 and released in the atmosphere is a loss of energy for the animal and may reduce the amount of energy used or retained by the animal. Information is scarce, however, on the direct relationship between the archaea methanogen community in the gut and the host's energy metabolism and body fat retention. A higher abundance and diversity of methanogens is sometimes correlated with a lower fat storage in lean compared to fat pig breeds. The biochemical pathways and physiological mechanisms involved require further clarification.

Among the two main methanogenic gena, *Methanobrevibacter* spp. (i.e. *M. smithii*) are more efficient for CH_4 formation than *Methanosphaera* spp. Consequently, the composition of the methanogen community influences the energy balance and the body fat retention in the pig as well as the quantity of methane produced. Other studies underline the direct cooperation between archaea and bacteria to obtain energy from short-chain polysaccharides in the diet.

For future CH_4 mitigation strategies, increasing the diversity of the methanogenic community towards a higher proportion of less efficient *Methanosphaera* spp. constitutes an approach for decreasing CH_4 formation. Similarly, adjusting the competition for H_2 uptake between acetogens and methanogens towards acetogenesis could help to decrease CH_4 formation. Stimulating acetogenesis is a more favourable direction for both the host and the environment per amount of feed ingested. However, adjusting the microbiota in the gut of pigs may also influence formation and absorption of SCFAs and energy utilization from the diet.

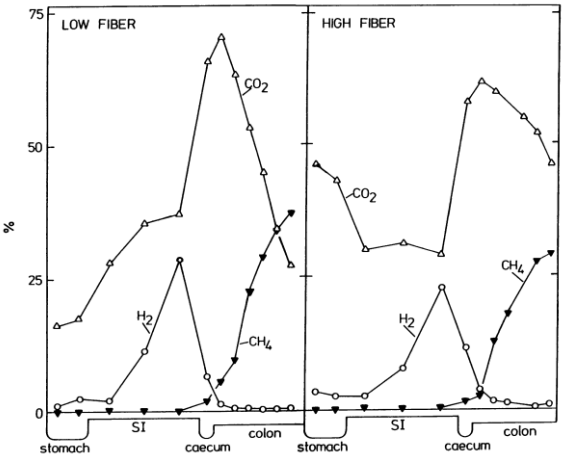
3 Quantitative evaluation of enteric formation of CH_4 in pigs

The current chapter presents an insight into formation of enteric methane in various segments of the digestive tract and how enteric gas formation can be measured. Existing data on the enteric gas formation of pigs mainly come from experiments conducted in respiration chambers where animals are kept for a few hours up to a few days in order to assess whole body energy metabolism. Regarding the utilization of dietary energy by animals in such setting, measurement of CH_4 and H_2 are important as they are combustible gases and represent a loss of energy for the animal (Jørgensen et al., 2011). Consequently, in these experiments, methane released by the animal into the air is measured. Using results of the energy metabolism studies, other authors have reviewed the impact of animal weight or age, or feed characteristics on CH_4 formation. As the level of enteric CH_4 formation is largely determined by the feed intake and fibre content of the diet and the fermentative capacity of the hindgut of pig, different authors proposed equations to quantify the daily methane formation in pigs taking into account factors as body weight, feed intake and dietary fibre intake, which are analysed or calculated in different ways.

3.1 Sites of the gut formation of gases

3.1.1 Composition of digestive gases

The fermentation of fibre in the gastro-intestinal tract of pigs results in the formation of short chain fatty acids, CO_2, H_2, CH_4, urea and heat. Information about the relative amounts of gas formation in different regions of the GIT in pigs was mainly provided by a study undertaken *in vivo* by Jensen and Jørgensen (1994). They collected and analysed gas samples taken from 12 subsequent segments along the gut of pigs. For H_2, results showed that low levels were found in gas from the stomach followed by an increase along the small intestine, as some fermentation of dietary fibre by microorganisms began in the stomach and small intestine. A maximum in the proportion of H_2 in total gas collected (± 25 % of total gas) was reached in the last third of the small intestine (Figure 10).



Low fibre diet based on wheat starch, barley, fish meal and casein. High fibre diet based on barley (57%) and pea fibre (35%). The residual gas is mainly composed of N_2 (not shown). Reproduced from: Jensen & Jørgensen, 1994.

Figure 10 *Gas composition (% of total gas collected) in the various gastro-intestinal segments of pigs fed with low or high fibre diets with 6 and 24 % dietary fibre / kg DM.*

The gas collected from the caecum and the proximal part of the colon also contained H_2 but not the distal segments of the large intestine, in spite that H_2 formation is an obligate part of anaerobic fermentation (reviewed by Jørgensen et al., 2011).

The gas from caecal digesta contained significantly higher amounts of H_2 for pigs given a high-fibre diet based on barley and pea fibre than for pigs given a low-fibre diet based on barley and wheat starch. The decrease of the H_2 concentration in the distal part of the large intestine could also result from the use of H_2 as electron donor in methanogenic or acetogenic fermentation. The pattern of the enteric CO_2 formation was also largely impacted by the dietary fibre concentration of the diet as shown in Figure 10. In the stomach, the percentage of CO_2 in total gas collected from the intestinal segments of the pigs receiving the high-fibre diet was much higher than that in the pigs receiving the low-fibre diet (45% versus 17%). For both groups of pigs, the percentage of CO_2 in the small intestine was around 30%, and reached 60 to 70%, in the caecum and first segment of the large intestine. This was followed by a steady decrease in the following segments, more pronounced in the pigs fed the low-fibre diet. Lastly, methane was not detected in stomach or small intestine of the pigs. Small amounts of CH_4 were found in gas from the caecum, followed by a steady increase in the colon, reaching concentrations as high as 29 to 37% in the rectum (Jensen & Jørgensen, 1994).

These results corroborate with the findings of studies on the microbiome of pigs showing that the composition and density of methanogens in the GIT of non-ruminants depend on the specific parts of the GIT (Murru et al., 2018). Seradj et al. (2018) showed that abundance of methanogens increased throughout the large intestine reflecting an improvement in the archaea fermentation conditions. Whereas in the caecum of pigs, the CH_4 formation is substantially variable and low (Robinson et al., 1989), CH_4 is likely to be produced distally in the colon of pigs. Indeed, Christensen and Thorbek (1987) using respirometry showed that the highest values of daily CH_4 formation were measured for pigs fed near *ad libitum* as a greater amount of non-enzymatically digested material in the small intestine reached the hind-gut. Using *in vitro* approaches to assess differences in fermentative activities of digesta obtained from various regions of the pig gastrointestinal tract, Robinson et al. (1989) indicated that the colon is the site showing the highest CH_4-forming activity. While total-gas formation per time per segment did not differ across the pig small intestine, caecum and colon, considerable differences were observed by Robinson et al. (1989) for the amounts of CH_4 formation per unit of time across regions of the porcine lower GIT.

3.1.2 Elimination of methane and other digestive gases

Gases produced by the gut microbiota during fermentation can be eliminated from the GIT via three pathways: _further metabolism by gas-consuming microorganisms into non-gaseous products (e.g., reductive acetogens converting H_2 and CO_2 to acetate); _ absorption via the mucosa into the bloodstream and exhalation via the breath; _elimination through the anus as flatulence (Mutuyemungu et al., 2023). A study by Mego et al. (2017) showed that in humans 22% of the microbial gas produced was evacuated through the anus, while the rest (78%) was eliminated via the alternative pathways. However, the rate at which gases can be eliminated via metabolism or blood absorption before reaching the anus is not static. In the study of Mego et al. (2017), after the start of the supply of prebiotic galactooligosaccharides (GOS), a rapidly fermenting non-digestible carbohydrate, the total gas volume in humans increased by 37 % after which the volume declined to baseline levels although the consumption of GOS continued for two weeks. Additionally, the proportion of total gas eliminated from the GIT before reaching the anus tended to increase slightly (78 to 87%) after a two-week administration the dietary treatment including GOS. Mutuyemungu et al. (2023) concluded that continued intake of fermentable ingredients may increase gas-consuming pathways by the microbiota and increase the ability of gas to be absorbed into the bloodstream and then eliminated in the breath.

For H_2, about one–third of the formation in the gut is reutilized by microorganisms in the colon, and a substantial part can be detected in breath and flatulence as reviewed by Pochart et al. (1992) and Mutuyemungu et al. (2023). Most CO_2 that is not metabolized by the gut microbiome is passively absorbed by the colonic mucosa, enters into circulation and is exhaled in breath (Christl et al., 1992). Unabsorbed CO_2 can be excreted as flatulence.

For methane, limited quantitative information is available on the routes of excretion of the intestinal produced CH_4 in pigs. In respiration chamber studies, the quantitative formation of CH_4 measured is the sum of CH_4 excreted via flatulence and via expired air. As CH_4 concentrations reach the highest levels in digesta in the distal colon and rectum, it can be speculated that a predominant part of CH_4 formation in pigs is excreted via flatulence. Interestingly, Mutuyemungu et al. (2023) underlined that the hydrogenotrophic methanogenesis decreases the gas partial pressure in the colon.

Indeed, the transformation of CO_2 and H_2 to CH_4 has the effect of reducing the total gas volume by a factor of 5, by consuming 4 moles of H_2 and 1 mole of CO_2 to produce 1 mole of CH_4 ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$). However, the volumes eliminated via the different ways may also be influenced by the extent of gut fermentation and methanogenesis. Whereas the abundance of methanogens in human faecal samples varies from undetectable to 10^9 cfu per g of faeces, results showed that a threshold population of 10^7-10^8 methanogenic archea /g dry weight faeces is required to result in detectable levels of CH_4 in the human breath (Miller and Wollin, 1986; Pochart et al., 1992). Consequently, whereas *M. Smithii* can be detected in the lower tract of 70% of the human population, only 15% has methane (≥3 ppm) in the breath (reviewed by Pimentel et al., 2013).

3.2 Methods to assess enteric methane formation

3.2.1 Measurements in respiration chambers

To date, little research has been specifically designed to study factors influencing enteric methane formation in pigs. To evaluate it, researchers have compiled data of the energy metabolism experiments carried out for non-lactating sows and growing pigs in respiration chambers. In most cases the measurements were part of feeding experiments evaluating the energy metabolism and utilization, including effects of feed and nutrient intake, and intake of different forms of fibre. From such experiments, quantitative CH_4 formation and factors affecting its formation could be evaluated.

In respiration chambers, the energy metabolism of pigs is calculated on the basis of heat production related to the consumption of oxygen (O_2) and formation of CO_2, CH_4 and H_2. Heat production can be estimated from the consumed amount of O_2 and produced amount of CO_2 and CH_4 (Christensen and Thorbek, 1987). For this, in climatic controlled airtight rooms, the atmospheric air is ventilated through the chambers and the amount of air is measured together with the concentration of O_2, CO_2, CH_4, and, in some studies H_2, in both in-going and out-going air (Jørgensen et al., 1996; Jørgensen et al., 2000). Typically, a nitrogen and energy balance experiment comprises a total period of about 12 days, including a 5-7 day period of adaptation of pigs to the diet and experimental conditions. Daily faeces and urine are collected quantitatively during the final 5-7 days. During this collection period, the metabolic cage with the pigs is placed in the respiration chamber with the amount of O_2 consumed and CH_4, CO_2 and H_2 produced during 2 x 24 h periods (Jørgensen et al., 2011). However, some respiration studies had simplified designs in which H_2 or CH_4 were not measured (Le Goff and Noblet, 2001; Noblet and Le Goff, 2001).

In Denmark, Jørgensen et al. (2011) compiled data of all experiments carried out in the respiration chambers at Research Centre Foulum during 20 years[7]. From a total of 16 experiments with growing pigs, 9 experiments with sows and one with piglets, these authors have established a dataset with data on the enteric gas formation for 140 different diets or other experimental treatments. The main results are described in paragraph 3.3 of this chapter. Data on CH_4 formation from similar studies mainly conducted in France and Germany have been collected by Vermorel et al. (2008), Dämmgen et al. (2012) and Philippe and Nicks (2015).

In the literature, CH_4 formation may be expressed as volume, mass or energy (litre, kg or MJ) per day and per animal. Jørgensen et al. (2011) underlined that not only the daily CH_4 formation per pig is of interest but also the formation relative to the amount of feed ingested (in kg, energy equivalents or dietary substrate available for fermentation). Consequently, authors used the following expressions:

1. Litre of CH_4 per day, which is calculated from the CH_4 concentration measured in the outgoing air.
2. Litre of CH_4 per kg dry matter (DM) or gross energy (GE) intake to take into account the potential amount of dietary substrate available for fermentation in the gut.
3. CH_4 formation per g of fermented fibre[8].
4. CH_4 formation per unit of digestible energy (DE) ingested.

[7] The old respiration chamber of Foulum was established in 1990. The construction of new chambers has been undertaken in 2020-21 then in 2023 at Viborg RC (Aarhus).

[8] Fraction of dietary fibre fermented primarily in the lower gut resulting in production of SCFA, CO_2, H_2, CH_4, urea and heat (Jørgensen et al., 2011).

For the conversion of volume to mass, the density of CH_4 is ρ=0.716 kg /m^3 at standard conditions[9]. For the conversion of mass to energy, the energy content of CH_4 is η= 55.65 MJ /kg.

3.2.2 Fermentation of digesta samples

To assess the differences in fermentative activities of digesta obtained from various regions of the pig GIT, Robinson et al. (1989) conducted an *in vitro* study in which digesta from the small intestine, caecum and colon of pigs were collected and incubated for 2 h or 5 h at 37 °C. Samples were analysed for total gas (gas pressure), CH_4, H_2, lactate, formate, acetate, propionate, butyrate, valerate, and isovalerate. The mean methanogenic and total-gas formation rate for the colon samples as cumulative ml per g of undiluted wet digesta and per hour of incubation were calculated. To evaluate the accuracy of their CH_4 formation estimates, Robinson et al. (1989) have compared their results with the data on CH_4 formation reported by Christensen and Thorbek (1987) for pigs fed a similar diet. As the latter authors indicated that an 85-kg pig could produce 5 to 7 litres of CH_4 / day, Robinson et al. (1989) have multiplied the mean CH_4 formation rate of 1.2 litres of CH_4 /kg of digesta per day by the average weight of luminal contents in the colon (1.0 kg), leading by extrapolation to a CH_4 formation rate of 1.2 litres of CH_4 per 85-kg pig per day. Robinson et al. (1989) concluded that this estimate was much lower than the CH_4 formation rate (5 to 7 litres of CH_4 per day) reported by Christensen and Thorbek (1987) for an 85-kg pig measured via a respiration chamber.

3.2.3 Methods using sensors or NIR analysis

Whereas use of a respiration chamber is considered the standard method, recent approaches or methods have been designed to assess the enteric methane emission at animal or barn scale in dairy farming. Some of these methods could be adapted to pig farms or used to update estimates for the annual enteric methane formation of livestock by country or region as part of the total GHG emission of animal production sectors.

Direct measurements of CH_4 concentration in the exhaled gasses can be undertaken trough the commercially available GreenFeed automated system (C-Lock, Rapid City, SD, USA) based on spot sampling of eructated and exhaled gases. The system delivers small amounts of pelleted feeds and allows multiple measurements per day on a large number of animals in farm conditions (Huhtanen et al., 2019; McGinn et al., 2021; Coppa et al., 2021).

The use of tracer techniques for measuring methane from ruminant has been in use for many years. Following early studies of isotopic techniques such as ^{3}H-methane or ^{14}C-methane, the sulphur hexafluoride (SF6) technique assists determination of enteric methane formation from large numbers of individual animals. A tube loaded with SF6 and with a calibrated release rate is inserted into the rumen of each animal prior to the experiment. As two gases from the rumen disperse identically into environment and SF6 tracer has a known release rate, the unknown CH_4 release rate may be estimated (Deighton et al., 2014; Berndt et al., 2014).

At the farm scale, a screening method for GHG emission in European dairy cattle barns is based on indoor and outdoor CO_2, CH_4, and N_2O concentration measurements and on a questionnaire developed to estimate the carbon mass balance at building level. It appeared that CH_4 emissions from manure could double those from the dairy enteric fermentation (Vergé et al., 2022). In Danish dairy herds, Thorup et al. (2022) simulated the effects of management strategies on enteric methane formation applying the SimHerd model, a module capable of estimating enteric methane formation from a dairy herd based on feed intake estimated for individual animals.

Vargas-Bello-Pérez et al. (2022) used a non-invasive sound technology to monitor rumen contractions and rumen gas fermentation. Using a wireless device (CURO MkII), authors showed that high quality recordings of rumen sounds were not only feasible, but they could also discern lactating from dry cows. It was indicated that there is now a need for focused research into the correlation of rumen sounds with measurements of rumen CH_4 and CO_2 in animals at different stages of production. Furthermore, methane formation in sheep could be predicted using computed tomography (CT) measurements of the volume of rumen (Lambe et al., 2019) as routine CT is used for scanning of sires in sheep breeding programmes.

[9] The German standard DIN 1343 uses a standard temperature T = 273.15 K (0 °C) and a standard pressure of 1013 hPa. Gas densities (ρ) can then be adjusted using the relation T1/T2 = ρ2/ρ1. (Dämmgen et al., 2012)

Various methodological protocols were evaluated by Coppa et al. (2022) to identify the best approach to predict enteric CH_4 formation using mid-infrared spectroscopy (MIR) on cow milk. Other results (Ferronato et al., 2019; Vanlierde et al. (2022) suggested the feasibility of having a proxy based on application of NIRS on faeces to estimate enteric CH_4 formation by dairy and beef cattle.

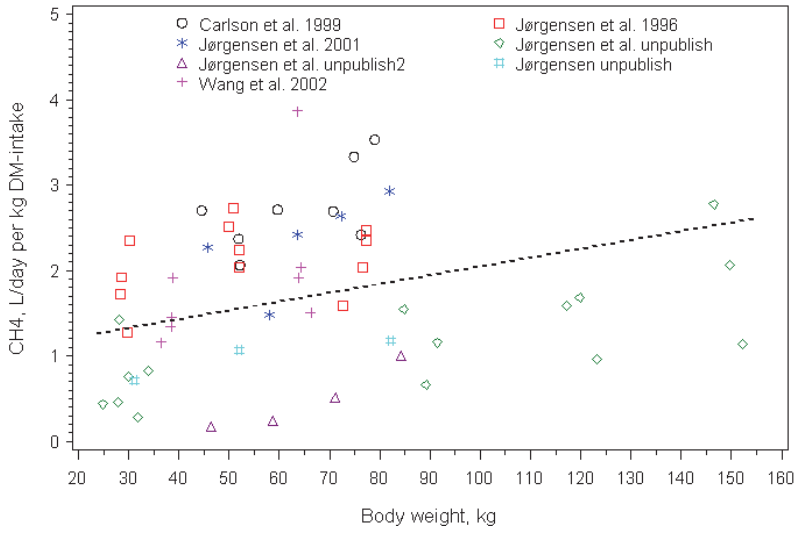
3.3 Evaluation of enteric methane formation by pigs

3.3.1 Influence of age and body weight on methane formation

A direct relationship can be found between the daily methane excretion and the body weight of herbivores (Clauss et al., 2020) or pigs (Le Goff et al., 2002a), as feed intake of domestic animals typically increases with a higher body weight. Additionally, the gastro-intestinal tract develops with age in pigs, resulting in a higher ability to ferment the fibre fraction of the diet. This can be explained by a larger microbiome in the gut, an increased capacity of the intestinal microbiome to ferment fibre, an increased transit time and a lower relative feeding level of heavier pigs (Le Goff et al., 2002a). Consequently, the daily formation of methane as measured in respiration chambers by Jørgensen et al. (2007) was more than 2.5 higher for sows with a body weight of 210 kg than for growing pigs in the weight range 60–115 kg (mean: 9.8 vs 3.4 L/d, respectively).

When calculated relative to the feed intake and feeding similar diets with equal fibre contents to pigs of different ages and body weights, a small effect of body weight on CH_4 formation was found by Jørgensen et al. (2011, Figure 11). This effect confirmed the higher ability of heavier pigs to ferment dietary fibre.

In formula, the relationship was described as enteric CH_4 formation (L /day per kg DM intake) = 1.01 + 0.0107 x BW (kg), n = 55, R^2= 0.71 (Jørgensen et al., 2011).



Reproduced from: Jørgensen et al., 2011.

Figure 11 *Influence of body weight on enteric formation of CH4 in pigs when corrected for feed intake.*

3.3.2 Effect of fibre content of the diet on enteric methane formation

The effect of the diet on the fermentation pattern in the lower gut of the pig is known for a long time (Kass et al., 1980; Stanogias and Pearce, 1985). Feeding high fibre diets increases the ileal flow of organic matter and reduces the digestibility of organic matter and nutrients compared to low fibre diets. Thus, feeding high fibre diets leads to a higher rate of non-enzymatically digested and non-absorbed substrates, mainly non-digestible carbohydrates, that reach the colon and may be fermented by the microbial system. Accordingly, respiration chamber studies of the past decades have shown a positive correlation between the fibre content in the diet and the amount of enteric CH_4 produced in pigs (Table 3).

Jensen and Jørgensen (1994) established that the release *in vivo* of CH_4 by fattening pigs fed a low fibre diet (5% NSP) was 1.4 L/day per animal (range 0.9 to 1.9 L), whereas a higher fibre diet based on 35% pea fibre (27% NSP) resulted in CH_4 formation of 12.5 L/day per animal (range 8.1 to 16.2 L). In a following study using similar low (0.9% soluble and 5% total NSP) and high (9% soluble and 26% total NSP) fibre diets, Jørgensen et al. (1996) obtained a six times higher CH_4 formation for the growing pigs given the high fibre diet compared to the low fibre diet (1.2 vs 0.2% of DE). It was also found from the sampling of intestinal contents that, for pigs fed diets with a high fibre content, fermentation may appear in the proximal hindgut, and methanogenesis may be more quantitatively important in the caecum (Robinson et al., 1989).

Accordingly, in a meta-analysis of respiratory chamber measurements, the enteric CH_4 formation appeared as positively correlated to the fibre concentrations (NSP, total fibre and total fermentable fibre) of the diet and negatively correlated to their protein, fat and starch contents (Jørgensen et al., 2011, Table 3). The highest correlation to the daily CH_4 formation was found for fermentable fibre per kg DM intake (r=0.86).

Table 3 *Correlations between the dietary nutrient content and the enteric formation of methane in pigs.*

	Protein	Fat	Starch	NSP	Total fibre	Total fibre	Fermentable fibre
	g/kg DM					g/d	
CH_4, L/d	-0.42	-0.23	-0.32	0.71	0.63	0.75	0.86
CO_2, L/d	-0.61	-0.47	0.18	0.33	0.34	0.65	0.62

Pearson correlations of the chemical composition of the diet (g/kg DM, g/d) and the formation of methane (CH_4) and carbon dioxide (CO_2) in L/d. NSP, non-starch polysaccharides. Total fibre, calculated as the residual fraction after subtraction of the analysed content of sugars, starch, crude protein, crude fat and ash from the dry matter content. Total fermentable fibre, the amount being fermented in the hind-gut. Results of 140 diets or treatments ($P<0.05$). From Jørgensen et al., 2011.

Moreover, the methanogenic activity is also stronger related to the content of soluble fibres. The inclusion (0 to 17%) of sugar beet pulp (SBP) in the diet of growing pigs resulted in increasing levels of NSP (up to 37 %) and linearly increased the CH_4 formation with a factor 2, in the study reported by Schrama et al. (1998). Accordingly, the highest CH_4 formation (0.63 vs on average 0.35% of digestible energy) was found for the growing pigs fed a diet based on sugar beet pulp (47 and 115 g/kg DM of insoluble and total NSP, respectively) compared with the control diet (9 and 43 g iNSP and NSP), the potato starch diet (9 and 40 g iNSP and NSP) and the wheat bran diet (14 and 107 g iNSP and NSP) in the study of Wang et al. (2004). In the study reported by Lee et al. (2022), growing pigs fed a high soluble fibre diet (25% NSP) based on pectin, potato pulp and sugar beet pulp, produced numerically but not significantly higher methane energy (0.35 vs 0.21-0.25 MJ/d) than pigs given a medium fibre diet (14% NSP) or a high-fibre diet using barley hulls (21% NSP) or a high insoluble fibre diet (24% NSP) based on brewers grains, pea hulls and grass seeds. Furthermore, the apparent ileal digestibility of NSP and of total carbohydrates (i.e. starch, NSP, sugar and fructans) was lower for the high soluble fibre diet than for other diets. Because of a high fermentation rate in the colon, however, the high soluble fibre diet exhibited a higher total tract digestibility of NSP and total carbohydrates and a lower faecal excretion of energy. Recently, Sattarova et al. (2022a,b) also reported higher enteric CH_4 formation in growing pigs for SBP than for wheat bran.

Studies using sows showed similarly that higher levels of fibre in the diet are associated with increased methane formation. Sows that were provided diets with a similar dietary fibre content (18 to 20% total dietary fibre) but different sources of fibre (Le Goff et al., 2002b) had a higher CH_4 formation for the diet in which maize bran replaced wheat bran (11.1 vs 7.4 L CH_4 / day, i.e. 1.3 vs 0.85% of DE), the SBP diet being intermediate (9.9 L/d corresponding to 1.2% of DE). As in growing pigs, these differences were related to differences in total tract digestibility of the fibre sources.

3.3.3 Influence of dietary protein and fat content and feeding level

From their meta-analysis, Jørgensen et al. (2011) found that there was a negative correlation between dietary fat or dietary protein content and enteric CH_4 formation. In two studies (Jørgensen et al., 1996; Jørgensen unpublished), growing pigs were fed diets with an increasing level of either rapeseed oil or fish oil. The concentration of the dietary fat varied from 3 to 21 % but had no significant effect on enteric CH_4 formation.

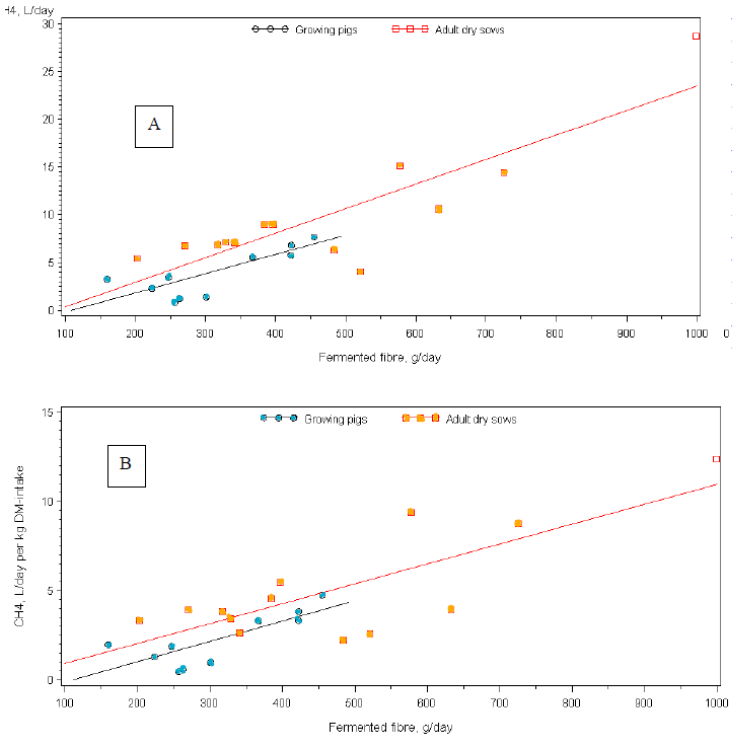
The authors underlined that this lack of effect of dietary fat on the CH_4 formation may be possibly due to the very high digestibility of the fat in the small intestine, resulting in absence of dietary fat in the hindgut.

In a study with restricted fed growing pigs, Christensen and Thorbek (1987) observed that feed restriction by 36% reduced CH_4 excretion by 23% compared with high feed level. However, related to DM intake, the pigs on low feed level excreted 3.1 litres CH_4/kg dietary DM and those on high feed level 2.5 litres, as a result that reduced feed intake allows more time for fermentation in GIT. This could also be the case when adult sows are fed relatively restricted (i.e.: 2-2.5 kg DM per day) in the dry period and during gestation.

3.3.4 Interaction effects of fermentative capacity and type of dietary fibre on methane formation

The level of enteric CH_4 formation is determined both by the content of the diet and by the fermentative capacity of the gut of pigs. As adult sows showed a higher capability than growing pigs to degrade rich-fibre sources, methanogenesis in the hindgut should be higher for sows than for pigs related to a same fibre intake. However, literature offers some contradictory results among authors. In their meta-analysis, Jørgensen et al. (2011) compared the ability of growing pigs and adult sows to utilise various fibre-rich feedstuffs. Including only experiments in which pigs and sows were fed the same type of fibre, results showed that growing pigs and sows produce the same amounts of CH_4 per g of fermented fibre (Figure 12).

In an additional analysis, Jørgensen et al. (2011) included all dietary treatments from the dataset, meaning that the sources of fibre were not identical for sows and pigs. The results showed a larger variation of CH_4 formation. However, the difference between growing pigs and adult sows was not significant, independently of the way methanogenesis was expressed (L/day or L/day/ per kg DM intake[10]).



Plot of daily CH_4 formation against total fermented fibre (g/kg Dry Matter intake) for growing pigs and adult sows in L/day (A) or when the formation is corrected for feed intake (L/day per kg DM intake (B). Reproduced from: Jørgensen et al., 2011.

Figure 12 *Comparison of daily CH4 formation by growing pigs and adult sows in experiments in which the animals were fed the same type of fibres.*

[10] When CH_4 emission is expressed relative to the DE, the slopes of the data reported by the latter authors were significantly different (N= 137, R2 = 0.72).
CH_4 energy, % DE = 0.0838 + 0.00376 (growing pigs) x Fermented Fibre (g/kg DM
CH_4 energy, % DE = 0.0838 + 0.00606 (adult sows) x Fermented Fibre (g /kg DM)

Besides that, other results showed that when high fibre diets are provided, the impact of the live weight appears as more important than with low fibre diets (Noblet and Shi, 1994; Jørgensen et al., 1996).

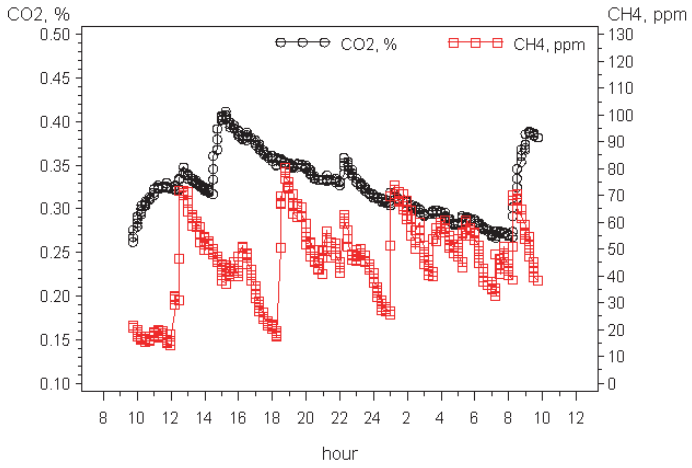
According to the data reviewed by Noblet and Le Goff (2001), the methane formation is about 0% of the digestible energy (DE) for piglets, 0.4% for growing pigs, 1% of DE for 120 kg pigs, 1.3% for sows but 3.4% for sows fed with high fibre diets. These results are in agreement with those of Jørgensen et al. (2011) who calculated CH_4 formation coefficient of 0.16±0.02%, 0.47±0.28% and 1.31±0.78% of DE for piglets, growing pigs and sows, respectively. However, for sows, Jørgensen et al. (2011) indicated a range of values from 0.40 to 3.25% of DE. Accordingly, Dämmgen et al. (2012) reviewed ranges of CH_4 formation from 0.2 to 1.1% of DE for growing pigs and from 0.5 to 3.4% of DE for sows.

Lastly, Jørgensen et al. (2007) reported that feeding diets rich in soluble fibre or with a high water holding capacity (based on SBP, potato pulp, pectin residue) to growing pigs and adult sows (77 and 110 g soluble NSP /kg DM, respectively) resulted in fermentation activity and CH_4 formation of both growing pigs (10 L CH_4/d and 1.4 % of DE) and sows (18 L/d and 2.7% of DE). On the other hand, adult sows showed a higher fermentative capacity and methane formation (13 L/d and 2.4% of DE) compared to growing pigs (6 L/d and 0.8% of DE), when high fibre diets with high amounts of insoluble fibre (from seed residue, pea hull, brewer's grain) were fed.

From the former, it can be concluded that adult sows have a higher capability than growing pigs to degrade fibre sources with a high lignin content (e.g. insoluble NSP). When diets contain more soluble NSP, the difference in fermentation capacity between growing pigs and adult sows becomes smaller.

3.3.5 Influence of environmental temperature and diurnal variation

Environmental conditions may influence the enteric methane and CO_2 formation according to Philippe and Nicks (2015) who mentioned influences of environmental temperature and diurnal variations. Jørgensen et al. (2011) underlined the large diurnal variation of CH_4 formation depending on feeding time and physical activity, and on digestion and fermentation processes in the intestinal tract (Figure 13). Whereas the variation in CO_2 formation is mainly a reflection of meal ingestion and related physical and metabolic activity, the sudden increase in CH_4 air concentration is related to occasional activity e.g. animals stand up and release intestinal gas via flatulence.
Additionally, using two experimental diets with a low fibre content (59, 9 and 52 g/kg DM of dietary fibre, soluble NSP and total NSP, respectively) or high fibre content (268, 93 and 256 g/kg DM, respectively) for feeding pigs, Jørgensen et al. (1996) showed that there was no effect of environmental temperature (13 vs 23°C) and negligible effects of the environmental temperature × dietary fibre interaction on the energy metabolism and enteric formation of CH_4 in pigs.



Carbon dioxide (CO_2) and methane (CH_4) in the outgoing air from the respiration chamber with a growing pig fed twice daily a diet containing sugar beet pulp. Reproduced from: Jørgensen et al., 2011.

Figure 13 *Methane and carbon dioxide formation in the gut of growing pigs measured over the day.*

3.4 Prediction and modelling of enteric methane formation by pigs

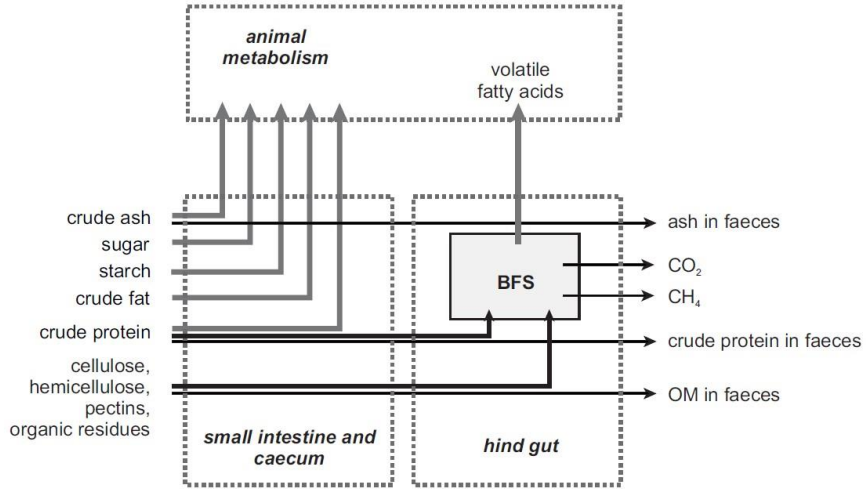
In the last decades, several research groups have derived simple functions to estimate the enteric methane formation of pigs, using the data of energy metabolism studies in respiration chambers. The integration of these functions with animal and diet characteristics are useful for mathematical modelling to predict enteric methane formation and evaluate mitigation approaches.

3.4.1 IPCC guidelines

Initially, the 1996 version of IPCC[11] guidelines proposed several methods for national greenhouse gas inventories. The Tier 2 method related CH_4 formation from enteric fermentation to the gross energy (GE) intake of pigs using a default methane conversion factor of 0.6 % for a mean feed intake of 38 MJ GE/pig/day for developed countries[12] (expressed as energy loss; IPCC, 1996[13]). This IPCC factor was initially established by Crutzen et al. (1986) who calculated a mean individual GE intake of 38 MJ/day for pig herds in Germany and used the 0.6% release measured by Schneider and Menke (1982) in a respiration chamber study with 80 kg pigs. In the 2006 version of IPCC, the evaluation was only made according to the simplified Tier 1 method. The enteric CH_4 formation was estimated at 1.5 kg /pig/year, corresponding to 4.1 g CH_4 per day (Dong et al., 2006[14]). This result is also based on the previous calculation made by Crutzen et al. (1986) for a enteric methane formation of 0.6% of 38 MJ GE intake/day/pig. However, feed intake and feed efficiency are more directly regulated by the net energy (NE) content of the diet than by the GE content. Moreover, Tier 1 and 2 methods do not take into account the influence of diet composition on fermentation processes and methane formation. Consequently, the IPPC approach is a poor estimate for all pig categories and does not seem adequate for modelling enteric methane formation in pigs.

3.4.2 Definition of the organic matter fermentable in the large intestine

In several countries, researchers simultaneously proposed to estimate the enteric methane formation from pigs on basis of the amount of organic matter from the diet fermented in the large intestine, in other words the fraction of digestible organic matter that is not hydrolysed and absorbed in the small intestine and can be fermented in the large intestine. This essentially corresponds to the fraction of digestible dietary fibre (e.g. mainly cellulose, hemicellulose and pectin) and digestible protein non-previously digested in the small intestine as indicated in Figure 14.



Gray arrows: share of constituents that can be resorbed. Wide black arrows: bacterially fermentable substrates; narrow black arrows: matter that is neither resorbable nor bacterially fermentable. Reproduced from: Dämmgen et al., 2012.

Figure 14 *Enzymatic digestion and fermentation of major constituents of the diet in the digestive tract of pigs.*

[11] Intergovernmental Panel on Climatic Change

[12] For developing countries, the emission factor is 1.3 % for a feed intake of 13 MJ/pig/day. Differences in emission factors of countries are driven by differences in feed intake and feed characteristic assumptions

[13] Table A-4 in IPPC, 1996

[14] Table 10.10, p.10.28 of IPPC 2006 (Dong et al., 2006)

This fraction of digestible dietary fibre was defined as "Bacterially fermentable substrates" (BFS[15]) in Germany (Kirchgessner et al., 1991; Dämmgen et al., 2012), as "Digestible residue" in France (Noblet et al., 2002) and as "fermented fibre" or "total fermentable fibre" in Denmark (Jørgensen et al., 2011). In the Netherlands, CVB (2022) has similarly proposed to group all polysaccharides degradable exclusively via fermentation under the term "Digestible Non-Starch Polysaccharides" (i.e. $DNSP_h$[16]).

It appears that all previous expressions correspond to a same definition of the organic matter fermented in the large intestine and can be expressed as follows[17]:

BFS or Digestible residue or Fermented fibre or $DNSP_h$ =
Digestible organic matter - Digestible crude protein - Digestible fat - Starch – Sugars (in g/kg)

where the digestible values of organic matter (OM), crude protein (CP) and Fat are based on total tract digestibility coefficients given in the national tables or systems for evaluation of feed ingredients. Figure 15 displays which chemically defined fractions in the diet account for the fermentable fibre fraction.

Table 4 *Concentration of crude fibre (CF), bacterially fermentable substrates (BFS) and metabolizable energy (ME) in different feed ingredients.*

Feedstuff	Crude Fibre g/kg	BFS g/kg	ME MJ/kg
Barley	46	75	12.5
Oats	98	60	11.1
Wheat bran	120	180	8.5
Dried sugar beet pulp	185	596	8.2
Green meal/cobs	180	270	7.4
Soya hulls	340	370	5.9
Malt germ	133	180	8.0
Apple pomace	195	260	7.4
Brewer's grains	160	175	8.0
Oat husk bran	230	117	5.6
Grass silage	180	330	6.5
Maize silage	165	203	8.6
Corn Cob Mix	46	80	13.0
Fibre mix	200	430	8.8
Straw	380	120	1.8

Adapted from: Lindermayer et al., 2009

The "BFS - Digestible residue" faction is a practical concept, used for the evaluation of the energy value of feed ingredients. It is involved as factor of equation of the ME value of feed ingredients in Germany (DLG Futterwertabellen, 2014) and of equation of the NE of feed ingredients in France (Noblet et al., 2002). It should be noted that all authors consider the protein fraction that is fermented by bacteria to be negligible (Figure 14). They do not take this fraction into account when calculating the fermentable organic matter fraction in the hindgut.

The BFS content of the single feed ingredients is given in German feed tables and the BFS value of a compound feed is the weighted mean of the BFS contents of its ingredients (Dämmgen et al., 2016). An example of the composition of feed ingredients for crude fibre, BFS and ME contents is shown in Table 4. Similarly, the digestible residue can be calculated from the OM, CP and fat contents and digestibility coefficients that are given in the INRA and AFZ Tables (Noblet et al. 2002) with different values for growing and adult pigs. The CVB Tables report the NSP_h digestibility coefficient for each ingredient obtained, just as for CP and CFATh, from digestibility studies[18].

[15] Or 'Bakteriell fermentierbare Substanz'

[16] NSPh and no longer NSP has been used since the CVB Livestock Feed Table 2016, because the new net energy system for fattening pigs introduced in 2015 (NE2015; EW2015) uses (V)RVETh and no longer RVET for all feedstuffs.

[17] A similar calculation method of BFS was given by Lindermayer et al. (2009) and Nehf et al. (2021) as follows:
BFS (g/kg) = Digestible crude fibre + Digestible nitrogen-free extract - Starch - Sugars (g/kg)

[18] In digestibility trials of feed ingredients, the digestibility coefficient of the NSPh fraction (DCNSPh) is always calculated according to the equation: DCNSPh = 100 x (DNSPh / NSPh) (contents in g per kg DM, DCNSPh in %).

Specific calculation of $DNSP_h$ in the Netherlands

More specifically, the equations published in the Netherlands by CVB (2022) for the calculation of the content of NSP_h and $DNSP_h$ take into account the content in the CVB table of sugars as glucose, and the presence of lactate, Glucose OligoSaccharides (GOS), glycerol and volatile substances (acetate, propionate, butyrate). Thus, the NSP_h fraction is calculated as follows:

NSP_h = OM - CP - FAT_h - $Starch_{am}$[19]– GOS - CF_DI[20]*sugars – 0.92*lactate – 0.5*(Acetate + Propionate + Butyrate) – Glycerol (contents in g per kg DM).

For the calculation of the content of digestible NSP_h, starch, GOS, sugars, lactate, glycerol and volatile substances are considered to be 100% digestible. Consequently, the equation is:

$DNSP_h$ = DOM - DCP - $DFAT_h$ - $Starch_{am}$ - GOS - CF_DI*SUG - Lactate - Acetate - Propionate - Butyrate - Glycerol (in g /kg DM).

However, for most dry feedstuffs and compounds feeds, the presence of lactate, GOS, glycerol and volatile substances is not taken into account[21]. Hence, for the calculation, simplified equations can be used for NSP_h and $DNSP_h$:

NSP_h = OM - CP - FAT_h - $Starch_{am}$ - CF_DI*SUG (in g /kg DM).

DNSPh = DOM - DCP - $DFAT_h$ - $Starch_{am}$ - CF_DI*SUG (in g /kg DM)

To conclude, definitions of fermentable organic matter fraction in the large intestine of pigs, published with different names in feed evaluation systems applied in Denmark, France, Germany and the Netherlands, are principally based on the same concept. The main differences among countries may be related to 1) the analytical methods applied as a reference to measure the chemical composition of feed ingredients, 2) the digestibility coefficients for OM, CP and Fat for each feed ingredient that are available in the national tables or online systems for the nutritional characterisation of feed ingredients.

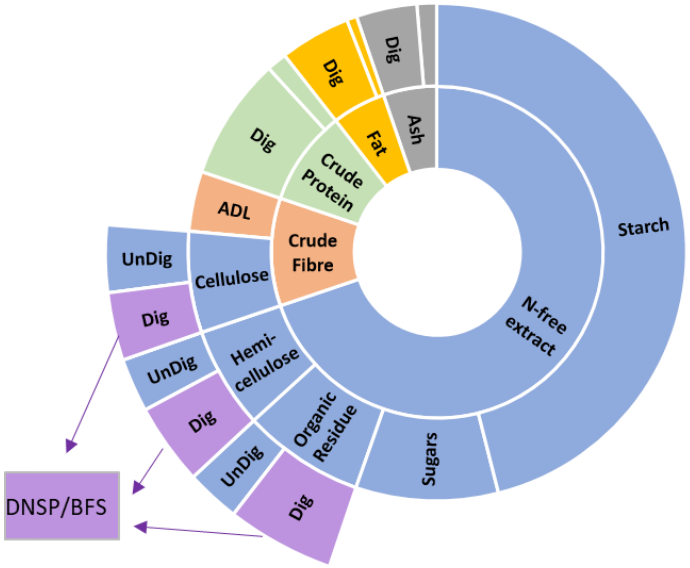


Figure 15 *Scheme depicting the calculated or analysed digestible NSP (DNSP) or Bacterially fermentable substrates (BFS) fraction of a diet or feed ingredient.*

[19] In the CVB Table, starch concentrations are indicated both for the enzymatic (amylo-glucosidase) method and the Ewers method.
[20] The factor CF_DI in the equation (and other equations) is the correction factor indicated in the CVB Tables for each ingredient, to convert the content of gross total sugars, expressed as glucose equivalents, into the sugar content as present in the product.
[21] For the feedstuffs as maize gluten feed and DDGS, also lactate and lactate + glycerol should be subtracted, respectively.

3.4.3 Prediction of the enteric methane formation

In Germany, France, Denmark and the Netherlands, using data from respiration chamber studies, researchers have proposed equations to relate the methane formation to the daily DNSP intake. These equations have been transformed in g of CH_4 per kg of daily intake of BFS or DNSP, and are displayed in Table 5[22].

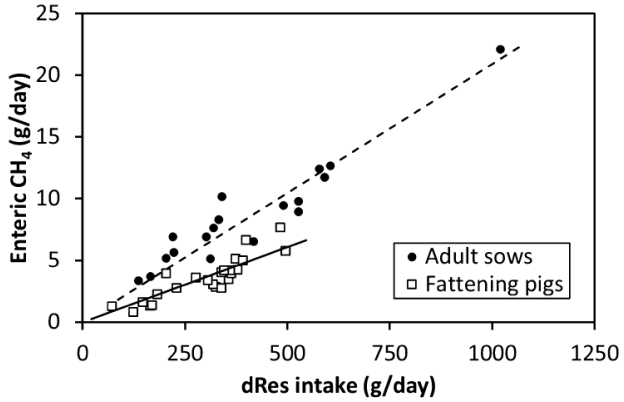
Table 5 *Equations proposed to estimate enteric CH4 formation by growing pigs and adult sows.*

Reference	a[1]	b[1]	CH_4 (g/kg diet with 15% DNSP)[2]	CH_4 (g per day)[3]
Growing pigs				
Kirchgessner et al. (1991)	0.0	0.020	3.0	6.0
Schrama et al. (1998)	-0.0647	0.0129	1.88	3.75
Vermorel et al. (2008)	0.0	0.012	1.80	3.60
Jørgensen et al. (2011)	-1.8788	0.01664	0.62	3.11
Jørgensen et al. (2011)	0.2952	0.01382	2.37	4.44
Philippe and Nicks (2015)	0.0	0.012	1.80	3.60
Adult sows				
Kirchgessner et al. (1991)	0.0029	0.013	1.95	5.86
Vermorel et al. (2008)	0.0	0.024	3.60	10.80
Jørgensen et al. (2011)	3.4624	0.00105	3.62	10.86
Philippe and Nicks (2015)	0.0	0.021	3.15	9.45

1 Coefficients of equation: CH_4 (g) = a + b × DNSP intake (g)

2 A 15% content of DNSP in the diet was adopted for the calculation

3 Calculation based on 300 g DNSP intake per day for growing pigs and 450 g for adult sows.



Source: Philippe and Nicks, 2015 adapted from Noblet et al., 1994; Jørgensen et al., 1996; Olesen and Jørgensen, 2001; Le Goff et al., 2002a,b; Ramonet et al., 2000; Galassi et al., 2004, 2005; Jørgensen et al., 2007; Serena et al., 2008. dRes: Digestible Residue, i.e. BFS or DNSP.

Figure 16 *Estimations of enteric CH4 formation by adult sows and fattening pigs as affected by the intake of BFS, DNSP and dRES.*

For a diet with 15 % BFS or DNSP, the use of equations of Schrama et al. (1998), Vermorel et al. (2008)[23] and Philippe and Nicks (2015) result in a very similar estimate for CH_4 formation in growing pigs, whereas the equation of Kirchgessner et al. (1991) results in higher values and the two equations of Jørgensen et al. (2011) result in lower or higher values.

[22] Original equations were determined as kg of CH_4 by kg of ingested BFS or Digestible Residue, by Kirchgessner et al. (1991), Vermorel et al. (2008) and Philippe and Nicks (2015). Jørgensen et al. (2011) proposed several equations of methanogenesis as L of CH_4 production related to feed intake or not, on basis of the fermented fibre intake. The equation published by Schrama et al. (1998) calculated kJ of CH_4 formed per kg of fNSP intake.

[23] Equations for growing pigs and sows published by Vermorel et al. (2008) were obtained from the compilation of data obtained in respiration chambers: Thesis of G. Le Goff (2001), unpublished data from J. Noblet, and results of Noblet and Van Milgen (2004).

For adult sows, a similar formation is found using the equations of Vermorel et al. (2008), Jørgensen et al. (2011) and Philippe and Nicks (2015), whereas use of the equation of Kirchgessner et al. (1991) leads to a lower estimation. From their meta-analysis of published data on enteric CH_4 formation according to the level of BFS or DNSP, Philippe and Nicks (2015) obtained distinct equations to predict the CH_4 enteric formation for fattening pigs and adult sows. As example, the ingestion of 300 g of DNSP is associated with the enteric formation of 3.6 g CH_4 by fattening pigs and 6.3 g CH_4 by adult sows (Figure 16).
A recent example of use is given by Hăbeanu et al. (2022), who applied the equation developed by Philippe and Nicks (2015) for growing pigs (See Table 5) to assess the quantity of methane produced by pigs fed different by-products of the oilseed crushing industry.

3.5 Conclusion

The enteric gas formation is for 99% composed of 5 gases: CO_2, H_2, N_2, O_2 and CH_4. H_2 concentration is the highest in the last third of the small intestine and CO_2 concentration reaches a maximum in the caecum and the first part of the large intestine. CH_4 formation is steadily increasing through the distal part of large intestine and reaches a maximum in the rectum. Consequently, the release in the air through the anus is thought to be higher for CH_4 than for the other gases. However, limited quantitative information is available on the relative contribution of excretion of enteric CH_4 via flatulence and via expired air in pigs. Measurements of concentrations of gases in a respiration chamber is the standard method for *in vivo* measuring of the enteric CH_4 formation but recently also methods using sensors or NIR analysis have been developed to assess the methane formation in the rumen of ruminants.

The enteric formation of methane in pigs is highly variable and depends mainly on the age or body weight of the animal, the feed intake and the fibre composition of the diet. A higher body weight generally indicates a higher ability to ferment the fibre fraction of the diet. Average estimates from the literature amount 0.8, 2.5 and 6 - 8 g CH_4 per animal per day for piglets, fattening pigs and adult sows, respectively.

The enteric CH_4 formation appeared positively correlated to the fibre concentration in the diet, and specifically to soluble fibre. Several authors have found that DNSP (also defined as Digestible Residue, Fermentable Fibre or BFS) was the best indicator of the amount of organic matter fermented in the colon by the microbiome. Consequently, several equations were proposed to estimate the enteric CH_4 formation by either growing pigs or adult sows related to the daily intake of DNSP. However, many *in vivo* and *in vitro* studies assessing the influence of the dietary fibre on the CH_4 formation used other parameters describing the fibre fraction as total carbohydrates, soluble, insoluble and total NSP, or total fibre. Some authors (Jørgensen et al., 2011; Dämmgen et al., 2012) concluded that still limited information is available on how and to what extent dietary ingredients, nutrient composition and animal body weight or age influence the quantitative formation of methane in the digestive tract of pigs.

4 Interventions to mitigate enteric CH_4 formation in pigs

There is a variety of strategies that have been developed for the reduction of enteric CH_4 formation by ruminants, including diet modifications, genetic selection of animals, microbiome manipulation in the digestive tract and the use of feed additives, such as plant secondary metabolites, methane inhibitors, and essential oils (Puente-Rodriguez and Groenestein, 2019; Hristov et al., 2022). Only a few studies have been undertaken to limit the enteric CH_4 formation in non-ruminants. Nevertheless, some approaches commonly used in ruminants may also find an application for non-ruminant animals (Misiukiewicz et al., 2021). This chapter aims to review interventions for the mitigation of enteric CH_4 formation by pigs.

4.1 Modification of the composition of the diet

Quantitative feed intake and nutrient composition of the diet have large effects on the fermentation pattern and on methane formation by archaea in the hindgut of nonruminant animals. As a consequence, adjustment of the nutrient composition of the diet (e.g. concentration of fibre, protein, and fat) may modulate the amount of substrate available for fermentation in the gut and the composition of the microbial community inhabiting the digestive tract of pigs, leading to a change of the enteric CH_4 formation. A number of studies have investigated the effects of modifying the concentration of a specific nutrient on enteric methane formation by pigs. However, when changing the nutrient composition of the diet, generally the level of other nutrients changes as well, which limits the possibility to draw firm conclusions. For instance, many protein sources are also high in fibre, whereas most energy sources (starch, oils and fats) are low in fibre.

4.1.1 Modulation of the dietary fibre content and impact of fibre-rich ingredients

As the formation of enteric CH_4 by pigs is strongly related to the dietary fibre content (see also 3.3.2), it may be assumed that lowering the fibre content of the diet allows to decrease the CH_4 enteric formation by pigs (Table 6). To illustrate this, low fibre diets (5 % total NSP) provided to fattening pigs resulted in 6 to 9 times lower enteric formation of CH_4 compared to high fibre diets with 26-27% total NSP in studies reported by Jensen and Jørgensen (1994) and Jørgensen et al. (1996). These authors tested extreme diets based on either starch, fish meal and casein (low fibre) or using 35% pea fibre (high fibre). As a practical reference, it should be considered that current diets manufactured for piglets or growing pigs in the Netherlands from wheat, maize, barley and soybean meal and some by-products from the cereal processing industry have a total NSP content ranging between 14 and 21%.

Several studies have shown that diets rich in soluble NSP resulted in a relative high enteric CH_4 formation (Kirchgessner et al., 1987; Jørgensen et al., 2007; Lee et al., 2022). Consequently, the inclusion of feed ingredients rich in soluble NSP such as SBP, potato pulp and other pectin containing ingredients should be limited in pig diets in order to reduce enteric methane formation. In particular SBP has a large stimulating effect on enteric CH_4 formation even at 12-15% inclusion levels (Wang et al., 2004; Sattarova et al., 2022a,b). Schrama et al. (1998) showed that CH_4 formation of growing pigs was increased 1.7 or 2.0 fold when using diets containing 5 or 15% SBP on DM basis, respectively, compared to a standard diet low in soluble NSP. It should be mentioned that sugar beet cultivation has slightly declined in Western Europe, resulting in a lower availability of pressed or wet SBP and lower inclusion levels of dried SBP in pig diets. Conversely, an ingredient such as wheat bran is rich in total NSP but has a higher ratio of insoluble relative to soluble NSP, compared to SBP. Consequently, a growing pig diet including 40% wheat bran replacing 15% SBP, having the same nutritional value, induced a lower enteric CH_4 formation (-25% per kg DM intake) in a study recently reported by Sattarova et al. (2022a,b). However, whereas the average DM intake of pigs was not influenced by diet in the latter study, the wheat bran diet decreased more than the SBP diet the apparent total tract digestibility of OM compared to the control diet (-5.8 and -3.4%, respectively).

Wang et al. (2004), likewise, previously found a 40% lower CH_4 formation in pigs fed a diet including 19 % wheat bran versus a diet with 12% SBP. Diets with high amounts of insoluble fibre (i.e. grain by-products, pea hull, brewer's grain) are more intensively fermented by adult sows than by growing pigs, resulting in a lower enteric CH_4 formation when allocated as feed ingredient to growing pigs (Jørgensen et al., 2007). It is important to note that differences in fermentation rates of these ingredients are related to lower net energy values for growing pigs than for sows as indicated by the digestibility coefficients of energy published by INRAE (2004) for wheat bran (57 and 63% for growing pigs and sows, respectively), and brewer's grain (52 and 58%, respectively).

Table 6 *Impact of the dietary fibre content on enteric CH4 formation in pigs as measured in respiration chambers.*

Reference	Ingredients	Total NSP	CH_4	Animal
		g/kg	L/pig/d	
Jensen & Jørgensen, 1994	0 vs 35% pea fibre		1.4 / 12.5	Finishers
Lee et al., 2022	0 vs 10% BH vs 18% Sol vs 21% Ins	138 / 206 / 247 / 239	1.2 / 1.0 / 1.6 / 1.0	Growers
Wang et al., 2004	0 vs 12% SBP vs 19% WB	43 / 116 / 107	2.3 / 5.7 / 3.2	Growers
Sattarova et al., 2023	0 vs 38 % WB vs 21% SBP	141 / 234 / 234	2.4 / 3.1 / 5.1	Growers
Sattarova et al., 2023	0 vs 40 % WB vs 22% SBP	146 / 256 / 243	4.9 / 6.1 / 9.3	G. sows*
Schrama et al., 1998	0 vs 5.5 vs 11 vs 17% SBP	275 / 305 / 334 / 365	2.4 / 4.1 / 4.3 / 4.9	Growers
Cao et al., 2013, 2016	21% WB vs 23% rice hulls	**	3.9 / 2.5	Growers

CH_4: methane, BH: barley hulls, SBP: sugar beet pulp, WB: wheat bran; Sol: 6% pectin residue +6% potato pulp + 6% SBP; Ins: 7% brewers grain +7 % pea hulls +7 % residue of ryegrass seeds; * gestating sows; ** 202 vs 330 g/kg NDF.

In fact, the capacity of pigs to ferment high fibre diets is also related to age and body weight of the animal and composition and activity of the microbiome in the GIT, which may result in differences in results among studies. For example, an unexpected higher CH_4 formation in growing pigs fed a diet with 21% wheat bran was observed compared to animals fed a diet with 23% rice hulls (Cao et al., 2013, 2016).

Overall, a limited number of studies are available which quantified the impact of dietary fibre content and composition on enteric CH_4 formation in nutrient balanced diets as used by commercial pig farms in Western Europe. Some trade-offs are to be expected from the requirement to produce a more sustainable pork meat using more fibre-rich and circular feed ingredients which could also result in a higher concentration of digestible NSP in the diet. As conclusion, regulating the quantity of dietary digestible NSP appears as one of the most promising solutions for reducing enteric formation of CH_4. More studies are required to quantify effects using diets which are nutritionally balanced, but vary in fibre content and composition. Further studies are needed to 1) estimate the amount of CH_4 produced using different commercial diets on basis of available equations and 2) check the accuracy of these estimations under *in vivo* conditions.

4.1.2 Reduction of the dietary protein content

The reduction of crude protein content in pig diets using supplementation strategies with free amino acids has been practiced for several decades by the pig sector to improve protein utilization and decrease N excretion and NH_3 emission by the pig farms, while maintaining or improving animal performance. As underlined by Philippe and Nicks (2015), it has also been assumed that lowering dietary crude protein content reduces CO_2 and CH_4 emission due to improved nutrient utilization and concomitant reduction in carbon excretion. The lower VFA production in the hindgut with a low crude protein diet could explain a lower enteric formation of CH_4 according to Velthof et al. (2005), but literature is contradictory on this point as reviewed by Philippe and Nicks (2015).

Regarding the effect of the reduction of the dietary crude protein content on the CH_4 formation by pigs or sows, authors have reported non-significant differences or increases in CH_4 formation as well as a reduction ranging from 13% under field conditions (Philippe et al., 2006) to 60% measured in respiratory chambers (Atakora et al., 2003a sows).

On the other hand, Jørgensen et al., 2011, found a negative correlation between enteric CH_4 formation and the protein content of the diet in a meta-analysis of studies performed in Danish respiratory chambers. It should be underlined that in most studies the reduction of the CP content of the pig diet was concomitant with a reduction of the fibre content.

In finishing pigs, Atakora et al. (2003b, 2005, 2011a) measured in respiration chambers that a reduction of the content in crude protein (from 19.5 to 16.5%) and neutral digestible fibre (NDF; 24.1 to 22.6%) in a barley-based diet, decreased CH_4 formation significantly (23.2 vs 17.6 g/d; -27%). However, the latter authors found that a larger reduction (to 12.0% CP and 16.5% NDF) only tended to further reduce the enteric CH_4 formation (to 17.0 g/d). Additionally, Atakora et al. (2011a) reported that a similar CP reduction for a diet based on corn grain (19.8 to 17.5% and 25.4 to 23.1% for CP and NDF, respectively) resulted in only a 6% reduction of CH_4 formation (25.4 to 23.9 g/d). These results corroborated the previous findings of the same group of authors with sows receiving either barley- or corn-based diets (Atakora et al., 2003a). Atakora et al. (2011a) underlined that, for the protein-rich corn-based diet, a higher level of dietary lysine increased feed efficiency and N, C and energy retention resulting in similar fluxes of nutrients passing to the hindgut and in similar N excretion and CH_4 formation.

In summary, it appears that the impact of CP and fibre contents in pig diets on enteric CH_4 formation cannot be separated as their levels are often interrelated in a pig diet. In other words, the reducing effect on CH_4 formation related to a decrease of the CP content of the diet may be a side effect of a lower fibre content of the diet.

4.1.3 Influence of the dietary lipid content

Numerous studies in ruminants have shown that supplementation of fatty acids, oils, and oilseeds in forage-based diets may diminish methanogenesis (Cieślak et al., 2013; Patra, 2013). Fat sources with medium- or long- chain fatty acids i.e. coconut oil and palm oil are also shown to depress CH_4 formation in ruminants (Machmüller, 2006). Beauchemin et al. (2020) reviewed that a supplementation of diets with lipids (<4% of dry matter intake) can decrease CH_4 formation in the rumen (by up to 20%) while benefiting animal productivity.

In the rumen, dietary lipids can potentially decrease the CH_4 formation by replacing rumen fermentable organic matter in the diet, decreasing the numbers of ruminal methanogens and protozoa, due to biohydrogenation of unsaturated fatty acids and enhanced propionate production (Patra, 2013). Propionate in contrast to acetate is not a direct substrate for methanogens. Biohydrogenation of e.g. unsaturated fatty acids can provide an alternative hydrogen sink in the rumen to compete with methanogenesis (Beauchemin et al., 2020). Although it is generally accepted that qualitatively fermentative processes in the hindgut of pigs are the same as in the rumen, these results obtained in ruminants cannot be considered relevant for pigs because fat is mainly hydrolysed and absorbed in the small intestine of non-ruminants, and does not pass to a large extent to the hindgut where most microbial fermentation takes place. Nonetheless, Jørgensen et al. (2011) reported a negative correlation between dietary fat and enteric CH_4 formation from their meta-analyse. A few studies may indicate that fat decreased microbial fermentation in non-ruminants. Less flatus in humans was produced from full-fat soya-bean meal than from defatted soya-bean meal (Steggerda et al., 1966). The inclusion of 350 g beef fat or safflower oil/kg in purified low-fat diet significantly reduced the number of caecal bacteria and decreased the microbial enzyme activities in the caecum of rats (Mallett et al., 1985). The only study in pigs was reported by Christensen and Thorbek (1987) who added 90 g/kg of soy-bean oil to the diet which reduced the amount of CH_4 enteric formation in pigs by an average of 26% compared to the control diet without oil (4.3 vs 5.2 L/ kg DM intake). No explanation was proposed by the latter authors about the mitigating effect of this extra energy on CH_4 formation. It may also be suggested that, with a same dietary energy concentration, increasing the fat content will lead to an increase in low-energy, high NSP ingredients, which might bring more soluble NSP and increase methane formation in the hindgut of pigs. Furthermore, although lipid supplementation may be implemented easily in compound pig diets, this may also increase cost and alter the fatty acid composition of meat (Grainger and Beauchemin, 2011; Patra, 2013).

In summary, little information is available on the effects of lipid concentration in pig diets on enteric methane formation.

4.1.4 Influence of feed or ingredient processing

Some specific technological processing of feeds or feed ingredients may modify the greenhouse gas and ammonia emissions from pigs during digestion and manure storage.

Feed processing (e.g. grinding, pelleting, and expansion) can increase nutrient digestibility of pig diets and consequently may reduce feed intake and enteric formation of CH_4. However, little quantified information is available. Two pig feeding experiments were undertaken by Dämmgen et al. (2016) to quantify the emission reduction potentials of GHG related to change in feed processing. However, total emissions from the digestive process and from manure management were hardly influenced by feed processing, with the exception of pelleting for which animal performance was improved and the fattening period was shortened. In this study, improvement of digestibility of nitrogen and OM from processing was measured but enteric CH_4 formation was only calculated based on diet composition and not measured.

The fermentation of rapeseed meal using bacteria and yeast (such as *Rhizopus oligosporus*, *Aspergillus oryzae*, or *Lactobacillus fermentum*) can reduce the level of aliphatic compounds, glucosinolates, oligosaccharides, lignin and NDF, and phytic acid (Bau et al., 1994; Shi et al., 2015) which could modulate GIT microbiota and mitigate methane formation. Recently, Gao et al. (2020) reported that replacement of soybean meal (SBM) or rapeseed meal (RSM) by 16% fermented RSM reduced both methanogen population and methane emission from the fermentation of caecal digesta (by 21% and 51%, respectively in 14-d old and 28-d old broiler chickens), without any unfavourable effects on growth performance. These results are in agreement with those of Cieślak et al. (2022) who found in batch culture studies and in *in vivo* experiments that providing 2.65 kg/d/cow of fermented rapeseed cake reduced the methane formation by 26 and 10% (Hohenheim Gas Test or batch culture, respectively) and the methane emission by 17 and 11% (experiments with cannulated cows or commercial dairy cows, respectively).

4.2 Influence of feed additives and specific ingredients

Among 246 nutritional substances or agents that could potentially reduce GHG emissions in the environment by livestock, Lewis et al. (2013) reviewed that 130 substances offer potential benefits. For non-ruminants, specific feed additives or ingredients containing specific functional compounds, such as probiotics, plant metabolites and organic acids have been studied and could be further investigated as mitigation strategies to reduce enteric methane formation in pigs.

4.2.1 Enzymes

Exogenous enzymes supplemented to the diet are key tools for improving nutrient efficiency and reducing environmental impact of pig production. Whereas the addition of xylanase to wheat based diets may improve nutrient digestibility and performance of pigs (Kim et al., 2005), the dietary inclusion of phytase in pig diets reduces P excretion (Jongbloed et al., 2000). Atakora et al. (2011b) investigated the effects of xylanase and phytase supplementation on energy metabolism and enteric methane in growing finishing pigs fed wheat based diets. The supplementation with phytase, xylanase, or combined phytase–xylanase increased digestibility of NDF and Acid Digestible Fibre (ADF) but did not affect C, N or energy balance and did not influence the enteric formation of CH_4 (18, 21 and 22 g/d/pig, respectively) compared to the control low protein (16.2%) diet (20 g/d).

4.2.2 Probiotics

Probiotic agents are believed to improve the microbial environment and composition in the gut, and may improve nutrient digestibility, growth performance and health status as a result (Liao and Nyachoti, 2017). In particular, yeasts and bacteria have been used as additives to support the microbiome composition in the gut and the growth performance of pigs (Vasquez et al., 2022; Zhu et al, 2022).

a) Yeasts

Yeasts provided via the diet may alter the fermentation pattern in the hindgut by decreasing acetate and increasing propionate production, and may decrease the density of *Methanobrevibacter* spp. in the colon of pigs (Gong et al., 2018). Some *in vitro* experiments have shown a positive effect of yeast culture and live yeast on mitigating CH_4 formation (O'Brien et al., 2014). Chaucheyras et al. (1995) investigated *in vitro* the effect of live yeast cells of *S. cerevisiae* on acetate and methane formation by two hydrogenotrophic microorganisms, an acetogen and a methanogen. They reported that yeast supplementation enhanced the hydrogen trophic metabolism of the acetogenic strain and its acetate production by more than fivefold, while in a mixed culture of acetogens and methanogens, without yeast supplementation, H_2 was mainly used for CH_4 formation.

For ruminants, several commercial microbial products are available, but their efficacy to mitigate CH_4 rumen formation varied among strains (Gong et al., 2018). For example, McGinn et al. (2004) used a commercial yeast product and reported a 3% decrease in CH_4 formation in beef cattle, whereas Chung et al. (2011) supplemented a novel *S. cerevisiae* to nonlactating Holstein cows and reported a 7% decrease in CH_4 formation.

For pigs, a dietary supplementation with *Saccharomyces cerevisiae* YST2 has shown to reduce enteric CH_4 formation *in vivo* in swine by 25% (3.0 vs 4.1 L/d) (Gong et al., 2018) which is consistent with the 10 to 25% decrease in CH_4 *in vitro* formation measured previously by the same group of authors (Gong et al., 2013). Interestingly, *S. cerevisiae* YST2 also decreased the pH from 6.99 to 6.69 in rectal digesta, and lowered the redox potential in caecum and colon in the study of Gong et al. (2018). The pigs fed the supplemented diet also had a lower acetate, and higher propionate molar proportion in digesta in the caecum and colon, and a decrease in *Methanobrevibacter* spp. in the upper colon, as well as an increase in the acetogen community along with lowering of the methanogenic activity in the caecum. According to Gong et al. (2018), low values of redox potential in the GIT may suggest O_2 removal in the GIT, creating a more anaerobic environment favourable for the anaerobic bacteria. This hypothesis is supported by Newbold et al. (1995) who reported a 46% to 89% increase in O_2 consumption in the rumen after diet supplementation with yeasts. The lower pH may reflect the degradation of carbohydrates and absorption of VFA. According to studies reviewed by Gong et al. (2018), a low pH of digesta indicates a higher production of propionate over acetate, which in turn decreases CH_4 formation. Lana et al. (1998) reported a lower ruminal CH_4 formation when the rumen pH decreased.

b) Bacteria

Hydrogen utilizing bacteria, such as the acetogens, can use hydrogen to produce acetic acid, providing an alternative route to eliminate hydrogen, in competition with methanogenic archaea. Numerous studies have shown that supplementation of pig diets with lactic acid bacteria modulates the colon microbiome and enteroendocrine cells in the intestinal mucosa of pigs but only a few studies investigated the effects on enteric gas formation. A commercial mixture (Biofermin S) of live lactic acid bacteria (*Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Enterococcus faecalis*) was supplemented to a piglet diet by Tsukahara et al. (2001) resulting in a reduction of 50 and 35% of CO_2 and CH_4 formed during *in vitro* incubation of digesta samples, respectively, whereas the formation of H_2S was increased. The methane formation was reduced by 50% in caecal digesta (53 vs 105 µl/g/h), by 38% in digesta of the centripetal turns of the spiral loop of colon (105 vs 170 µl/g/h) and by 19% in digesta from the centrifugal turns of the spiral loop of colon (124 vs 153 µl/g/h). A decrease in acetate : propionate ratio was also observed in digesta from the caecum and the colon, which was likely related to the reduction of CO_2 formed, as CO_2 is produced from oxidative decarboxylation of pyruvate to acetyl-CoA and acetate. The relative increase of propionate may be related to the lactate formation by lactic acid bacteria, because part of lactate is metabolized to propionate by acid-utilizing bacteria (See Appendix 2). Philippe and Nicks (2015) noticed no other studies in their review and suggested that additional *in vivo* experiments need to be carried out to confirm the previous mentioned results on a larger scale.

4.2.3 Biologically active plant metabolites

Biologically active plant metabolites such as tannins, saponins and essential oils, have been extensively studied as strategies to reduce enteric methane formation in ruminants, and may also have a potential to suppress the growth or activity of methanogenic microorganisms in non-ruminants. Cieślak et al. (2013) have reviewed a large number of studies assessing the effects of these phytochemicals on methane formation and methanogen and protozoa populations in the rumen.

a) Saponins

Saponins (or triterpene glycosides) are found in many plants such as tea, *Yucca schidigera* and *Quillaja saponaria* and have been used for years in animal nutrition as feed additives. These compounds could act as promising natural constituents for reducing enteric methane formation. However, studies reported that addition of saponins to the diet results in rather variable responses regarding methane formation in the rumen and its excretion in environmental air. The effects of saponins in reducing CH_4 may be due to the reduction of protozoa (i.e. single-celled eukaryotes) and possibly methanogenic archaea (i.e. single-celled prokaryotes) (Jayanegara et al., 2014; Palangi and Lackner, 2022). Due to their structure, saponins can interact with cholesterol present in eukaryotic cell membranes and cause destruction of this cell type (Cheeke, 1996; Wina et al., 2005). This may explain also a lack of direct effect of saponins on methanogenic archaea. Cieslak et al. (2013) reviewed that low saponin concentrations indirectly influence ruminal CH_4 formation by reducing the number of protozoa. As hydrogen is a key element involved in ruminal CH_4 formation, a lower number of protozoa, as hydrogen producers, can reduce CH_4 formation. Nevertheless, higher saponin concentrations may also have a direct negative effect on methanogens.

A meta-analysis of the effects of saponin-rich sources on ruminal methane formation through *in vitro* experiments showed that saponin-rich sources decreased methane formation per unit of substrate incubated as well as per unit of total gas produced, and also reduced acetate and increased propionate in the total VFA fraction (Jayanegara et al., 2014). Authors underlined that methane mitigating properties of saponins in the rumen are level- and source-dependent. Confirmation of saponin affecting methanogenesis was given by Wina et al. (2005), which used saponin containing extract of *Sapindus rarak*, by Wang et al. (1998) using *Yucca schidigera* extract, and by Hess et al. (2005) using a diet containing saponin from *Sapindus saponaria*.

In a recent review, Palangi and Lackner (2022) indicated that the results of *in vivo* studies with sheep or cattle provide contrasting results with some experiments showing a reduction in methane formation whereas others reported no significant reduction, considering studies based on the supplementation of extracts or powder of saponin-rich plants. Particularly, no significant effects in dairy cows were found from the addition of *Yucca schidigera* powder or *Quillaja saponaria* powder (Holtshausen et al., 2009; van Zijderveld et al., 2011). The results of Holtshausen et al. (2009) showed that saponin from *Y. schidigera* and *Q. Saponaria* lowered methane formation from dairy cows, but that the reduction was due to reduced ruminal fermentation and lower feed digestibility. These authors also found that feeding a lower dose of saponin to lactating dairy cows avoided negative effects on ruminal fermentation and feed digestion, but methane formation was not reduced. On the other hand, addition of tea saponin to sheep diets reduced methane emissions but did not significantly reduced the acetate : propionate ratio in the *in vivo* studies reported by Yuan et al. (2007) and Mao et al. (2010). The latter authors found that the counts of methanogens in the rumen were similar whereas saponin had a significant action against the protozoa.

Alfalfa meal is also rich in saponins and may inhibit caecal methanogenesis in birds. In spite of a high fibre content, the inclusion of 30% alfalfa meal resulted in a reduction of CH_4 formation in caecal digesta of mule ducks, Muscovy ducks, and geese by up to 67, 63, and 96%, respectively (Chen et al., 2014). The saponin content in the grower diet was calculated at 0.55%, based on a saponin content of 1.46% in selected alfalfa meal and 2.37% in unselected alfalfa meal as published before by Pond and Maner (1984).

The effect of saponin supplementation has been extensively studied in ruminants, but due to mechanisms involved it is not possible to extrapolate results to effects on enteric methanogenesis by pigs, and no specific information has been found on effects of saponins on enteric CH_4 in pigs. Additionally, difficulties for interpretation of experimental studies on ruminants are related to the lack of standardization of plant materials and extracts and to the unknown composition of the rest of plant extract products other than steroidal saponins (Cieślak et al., 2013). Lastly, effects of saponins have been reported to be transitory due to the deglycosylation of saponins to sapogenins by rumen bacteria (Wallace et al., 2001).

b) Other plant extracts

Other plants and plant extracts may show potential to inhibit enteric methane formation by animals, in addition to other effects on nutrient metabolism, health and performance. For ruminants, investigations on the effects of *Ericaceae* (e.g. blueberry), anise, capsicum, thyme, mint, orange oil, acacia, grape seed, green tea and other plant extract and essential oils have been reviewed by Palangi and Lackner (2022). *In vitro*, effects on rumen microbiota of *thymus capitatus* essential oil (and compounds) have been reported by Gini et al. (2022). Interestingly, alkaloids, kaempferol, quercetin, neochlorogenic acid and feruloylquinic acid were noted to reduce *in vitro* formation of methane, and were directly related to the yields of the solvent extraction process used to obtain these extracts from the plants (Ibrahim and Hassen, 2022). In addition, a few *in vivo* studies in ruminants have recently been undertaken. In the study of Stefenoni et al. (2021), oregano had no effect on CH_4 emission or lactation performance of the cows. Hart et al. (2019) found that a mixture of essential oils decreased CH_4 formation and increased milk yield of dairy cows. Lastly, Pedraza-Hernández et al. (2019) reported a decrease in CH_4 and CO_2 formation in goats fed with diets containing *M. oleifera* (i.e. moringa) extract and *S. cerevisiae*.

c) polyphenols

Two polyphenols (i.e. tannins), ellagic acid and gallic acid were reported by Manoni et al. (2023) to mitigate gas formation in an *in vitro* model of rumen fermentation. In this study, ellagic acid and ellagic acid plus gallic acid treatments decreased CH_4 formation by 20 and 25% per kg DM intake, respectively. Accordingly, polyphenols in apple pomace have been shown to decrease CH_4 formation from dairy cows by 8%, measured using respiration chambers (Cieślak et al., 2022). The authors also reported an increase in the population of bacteria and a decrease by 19% in the population of methanogens in ruminal fluid, as well as an increase in propionate and a decrease in acetate.

4.2.4 Algae

Machado et al. (2014) have evaluated the effects of 20 species of macroalgae for reducing *in vitro* methanogenesis of rumen fluid. All species reduced CH_4 formation and the brown seaweed *Dictyota* and the red seaweed *Asparagopsis* had the strongest effects, inhibiting CH_4 formation by 92% and 99% after 72 h, respectively. Both species also resulted in lower VFA and higher propionate concentrations in rumen fluid, indicating that anaerobic fermentation in the system was affected. These results are in agreements with those of Soares et al. (2022) who found that different samples of *A. taxiformis* applied at a bromoform ($CHBr_3$) dosage of 0.06 mg/g DM reduced CH_4 formation by 84 to 96% relative to the control treatment using an *in vitro* rumen fermentation system. For ruminants, a high activity of the genus *Asparagopsis* on the methanogenesis was also found in *in vivo* studies using respiration chambers (Li et al., 2018; Kinley et al., 2020) or connected sensor systems (Roque et al., 2019; Stefenoni et al., 2021).

Bromoform is the most abundant constituent with potential bioactive effects in *Asparagopsis* (Glasson et al., 2022). Halogenated alkanes as bromoform react competitively with the substrates of coenzyme M transferase and methyl-coenzyme M reductase, inhibiting methyl transfer from CH_3-H_4MPT to coenzyme M (CoM-SH), and the reductive release of methane from methyl-coenzyme M (CH_3-S-CoM). The availability of *Asparagopsis*, however, is limited and there are some concerns about the sustainability of its cultivation and potential negative effects of bromoform on animal health and rumen functionality (Min et al., 2021). For pigs, CoM-SH and CH_3-S-CoM are also essential final intermediates in the three metabolic pathways of the methane formation. However, although seaweed extracts were shown to have effects on the colon microbiome (Leonard et al., 2011), no studies were found about potential effects of algae on hindgut fermentation.

4.2.5 Methane inhibitors

a) 3-Nitrooxypropanol

3-Nitrooxypropanol (3-NOP) is currently one of the most promising solutions for the mitigation of methane emission by dairy cows (Almeida et al., 2022; Hristov et al., 2022) and beef cattle (Romero-Perez et al., 2014).

A commercial product (Bovaer®, DSM) containing minimum 10% of the active 3-NOP ingredient is now authorized for the cattle market in the EU. A large number of peer-viewed scientific studies have been published (Kindermann et al., 2019)

A recent *in vivo* study in The Netherlands showed that milk yield and DM intake were not impacted whereas CH_4 production was reduced by 19% for a total year when 3-NOP was included in the diet (Van Gastelen et al., 2022). Preliminary results in a Danish commercial farm also showed a reduction of enteric methane by 35% when 3-NOP was included in the diet (Nielsen et al., 2022).

The mode of action leading to inhibition of CH_4 formation during the last step of the methanogenesis pathway in rumen methanogenic archaea has been summarized by Yu et al. (2021). The molecular shape of 3-NOP is similar to that of methyl-coenzyme M, the co-factor involved in methyl transfer during methanogenesis. 3-NOP specifically binds into methyl-coenzyme M reductase (MCR), a nickel enzyme which has to be in the Ni(I) oxidation state for the enzyme to be active and to catalyse the CH_4-forming step in rumen fermentation. 3-NOP is a small molecule of low toxicity, highly soluble and rapidly metabolized in the rumen to low concentrations of nitrate, nitrite and 1,3-propanediol. The latter compound is further transformed into 3-hydroxypropionic acid (HPA), which is further used by mammalian cells as substrate for synthesis of acetyl-CoA and propanoyl-CoA. The latter serves as substrate for gluconeogenesis and is beneficial for lactating ruminants. Currently, 1,2-propanediol, also called propylene glycol, is allowed to feed to dairy cows and ewes as an alternative to reduce the negative energy balance generated by the high milk production in early lactation (Nielsen and Ingvartsen, 2004; Santos et al., 2017).

Using 3-NOP in the diet increased rumen H_2 formation and resulted in changes in the microbiome decreasing the relative abundance of *Methanobrevibacter* and increasing the relative abundance of Bacteroidetes (Gruninger et al., 2022). Interestingly, 3-NOP and canola oil reduced rumen CH_4 emission distinctly (-28% and -24%, respectively), and in combination (-51%) in the study reported by Gruninger et al. (2022).

To our knowledge, no study has been undertaken on the use of this additive in diets for pigs for reducing enteric methane formation in the hindgut. However as 3-NOP acts on the enzyme responsible for methane formation, the effect, when 3-NOP reaches the colon, should be similar to that observed in the rumen of ruminants.

b) Organic acids

Organic acids are commonly used in the diets of pigs as a means to influence the GIT microbiome and enhance health of the digestive tract. No common mode of action is described for individual organic acids and their salts and a number of different pathways are involved (Partanen and Jalava, 2005; Lewis et al., 2013). However, the general effect is that they reduce the pH and buffering capacity of the diet and digesta which together with their antimicrobial properties, help to prevent the growth of adverse bacteria such as *Salmonella* spp. and pathogenic *E. coli* in the gut, and improve enzymatic degradation and absorption of nutrients.

In pigs, organic acids differ in their ability to modulate fermentation in the digestive tract. Partanen and Jalava (2005) incubated *in vitro* several organic acids or salts and found that formic acid was the only acid that reduced the maximum rate of total gas formation. Concentrations of total VFA, acetate and propionate were reduced by formic acid, potassium sorbate and sodium benzoate compared to the control treatment, but ammonia and lactate concentrations were unaffected. Consequently, it could be assumed that the methanogenesis could also be influenced as a result of the lower formation of acetate in the colon when dietary organic acids are present. However, although some effects of organic acids may appear on pH and concentrations of VFA and bacteria in the caecum-colon (Suiryanrayna and Ramana, 2015; Tugnoli et al., 2020), organic acids are largely absorbed in the small intestine and generally do not reach the hindgut.

Protecting organic acids with encapsulation techniques can provide target-delivering organic acids along the small and large intestine (Tugnoli et al., 2020).

No additional *in vivo* or *in vitro* data have been published on the effects of organic acids on enteric methane formation in pigs. In birds, results of Chen et al. (2009) have shown that, surprisingly, formic acid inoculated into the oesophagus of geese was quickly converted into CH_4 as measured in respiration chambers, whereas acetate introduced into the caeca did not increase CH_4 formation, but conversely, tended to decrease CH_4 formation.

For fumaric acid or its salt, Lewis et al. (2013) reviewed that studies showing *in vivo* or *in vitro* a reduction of CH_4 formation were only conducted in ruminants, whereas the single study conducted in pigs focussed only on ammonia formation. Recently, Palangi and Macit (2021) showed that fumaric acid was more efficient than other organic acids to decrease the amount of CH_4 in incubation studies with rumen juice. Hence, fumaric acid could be employed in the diet to diminish CH_4 emission and to increase energy efficiency in ruminants. However, in a Dutch study on dairy cows, no significant effects were found from fumaric salt in the diet (van Zijderveld et al., 2011) emphasizing the need to confirm in *in vivo* studies effects on CH_4 reduction as observed *in vitro*.

Lewis et al. (2013) reviewed the effects of the inclusion in pig diets of benzoic acid or its sodium salt reported by seven studies, and all but one used an *in vivo* approach. All studies except one demonstrated a reduction in ammonia emission in exhausted air or from pig excreta with a mean reduction of around 35% across diets and concentrations. Enteric CH_4 formation was not evaluated in these studies. In the study reported by Aarnink et al. (2008), the dietary inclusion of benzoic acid reduced on average by 16% the ammonia emission in the in-house air of four Dutch farms, whereas no effect was found on methane emission.

c) Antibiotics

Although using antibiotics for controlling enteric fermentation and methanogenesis is undesirable today because of the need of a prudent and low use of antibiotics, their effects on microbial communities may help to understand potential mechanisms for mitigating methanogenesis. In the 1990s, several studies have investigated the capacity of different antibiotics to reduce methanogenesis in animals. Ionophore antibiotics (maduramicin, monensin, lasalocid, and salinomycin; also used as anticoccidials) usually reduce CH_4 in the rumen by decreasing H_2 formation, but in an *in vitro* study of Marounek et al. (1997), ionophores stimulated caecal methanogenesis in rabbits. The authors suggested that ionophores perhaps inhibit the H_2-dependent formation of acetate, leading to an increase in H_2 available for methanogens.

In their study, Piattoni et al. (1998) found that incubation of caecal digesta of fasted rabbits with bromoethansulfonic acid decreased CH_4 by 14% without altering the fermentation pattern, whereas monensin decreased CH_4 by 51%, and decreased total VFA production of 29%, mainly butyrate and acetate. However, bromoethansulfonic acid decreased CH_4 noticeably by 93% in non-fasted rabbits, while monensin increased CH_4 by 56% and led to a decreased concentration of total VFA of 16%, mainly via butyrate. As the supplementation of monensin did not decrease acetogenic bacteria, the increase in CH_4 was suggested to be due to a depression of autotrophic activity, leading to more H_2 being available for methanogenesis.

In addition, different in-feed antibiotics (e.g. avoparcin, bacitracin, lincomycin, spiramycin, tylosin, and virginiamycin) and dietary substrates (lactose, raffinose, starch, inulin, pectin, xylan, and cellulose) did not affect CH_4 formation in caecal digesta of chickens, and only altered mildly the pattern of fermentation products (Marounek et al., 1999).

4.3 Breeding strategies to reduce enteric methane

It has been indicated in Chapter 2 that the number and composition of methanogens in digesta and faeces can differ between breeds of pigs. However, there is very little information about the effects of pig breed, genetic line or gender on enteric methane formation in animals. For dairy cattle, several recent projects have indicated that breeding strategies may contribute to reduce enteric methane formation (Van Breukelen et al., 2022, 2023; Fresco et al., 2023). Strong genetic correlations were found between data on the composition of the core microbiota in the rumen and traits related to methane formation (González-Recio et al., 2022; Rowe et al., 2022). Positive heritability estimates were found for daily methane formation, methane yield and methane intensity[24] (Oliveira et al., 2022). Consequently, this information could lead to breeding strategies for low methane-emitting dairy cows (Oliveira et al., 2022; Manzanilla-Pech et al., 2022; Roehe et al., 2022).

In pigs, effects of genetic selection on environmental impact have mainly been related to the efficiency of N utilization and emission of NH_3 (reviewed by Philippe et al., 2011). Genetic lines with high growth performance and efficient protein deposition rate are related to reduced N output and a lower NH_3 emission. Similarly, a reduction of NH_3 emission is expected for boars compared to females and barrows because of their higher genetic capacity for deposition of body protein. In the future, genetic selection in pigs may also include new traits related to modulation of enteric methane formation. As example, Déru et al. (2020) found recently that total tract digestibility of nutrients was higher heritable in pigs fed a high fibre diet than a low fibre diet, and could be an interesting trait to include in future breeding objectives if pigs are fed with high fibre diets. Further studies of the same authors indicated that genetic by diet interactions on gut microbiota composition of growing pigs were limited (Déru et al, 2022a) and that the microbiota explained a significant proportion of the phenotypic variance of the digestive efficiency traits, even larger than that explained by the host genetics (Déru et al, 2022b). Interestingly, the proportion of phenotypic variance explained by the microbiota for some digestive efficiency traits was significantly higher under the high-fibre diet than under the conventional diet. Accordingly, the microbiota is a relevant source of information to improve the selection of digestive efficiency traits (Déru et al, 2024).

4.4 Conclusion

The formation of enteric CH_4 by pigs is directly related to the fibre content of the diet. Research has shown that the amount of organic matter that can be fermented in the colon (i.e. digestible NSP) is substrate for methanogenesis by archaea in the colon, and that decreasing the dietary (fermentable) NSP content reduces enteric CH_4 formation. Reducing the crude protein content or increasing the fat content may also result in mitigation of the enteric CH_4 formation as shown in studies in ruminants or non-ruminants, but effects are often confounded with changes in carbohydrate content and composition as well. However, results on the application of specific interventions on commercial pig diets to limit enteric CH_4 formation in farm conditions are only scarcely available. Interactions between dietary fibre, protein and fat contents and composition of the host microbiota in the GIT need to be taken into account. Furthermore, it is essential to quantify the impact of any change in the digestive process on all GHG produced in the digestive tract and from manure (i.e. CH_4, CO_2, NH_3, H_2, H_2S), as well as the potential consequence on the digestibility and utilization efficiency of N and P. An integrated approach is needed to take into account the consequences of changes in diet composition on the growth performance and health of pigs, on production costs, and on the circularity and sustainability of pig diets and as well as impacts on manure characteristics.

Most of the feed additives and plant extracts with potential effects on methanogenesis were demonstrated to have activity in *in vitro* research and when tested at relatively high dose levels. Whereas some studies in ruminants are available, only two studies were found that investigated *in vivo* the dietary supplementation of yeasts or lactic acid bacteria on the enteric CH_4 formation in pigs (Tsukahara et al., 2001; Gong et al., 2018). Additionally, results obtained in rumen targeted studies cannot be fully extrapolated to the pig colon because fermentation conditions and substrates available are not similar, particularly in relation to the fat content, pH and redox potential. Moreover, dietary supplements may be degraded and/or absorbed along the digestive tract prior to the large intestine, where most methane production takes place.

[24] Expressed as g CH_4 /kg fat- and protein-corrected milk

Literature suggests that saponins mitigate methanogenesis mainly by reducing the number of protozoa, condensed polyphenols (i.e. tannins) both by reducing the number of protozoa and by a direct toxic effect on methanogens, whereas plant extracts and essential oils act mostly by a direct toxic effect on methanogens. Constituents of algae also influence the relative ratio of VFAs and the acetate : propionate ratio. Methane inhibitors such as 3-NOP and organic acids show potential for reducing CH_4 formation in the rumen but only little information is available on methane formation in non-ruminants.

Many authors underlined that scientific information from long-term *in vivo* trials focussing on dietary intervention to reduce enteric methane formation is limited. Benefits on CH_4 formation associated with bioactive components *in vitro* may not always be obtained *in vivo* or could be lower over time due to in time adaptation of microbial communities involved in fermentation processes in the digestive tract. Indeed, as the rumen and colon of animals hold dynamic ecosystems, the investigation of the influence of plant metabolites or other feed additives on microorganisms involved in the process of methanogenesis should take into account factors that can neutralize the biological properties of these compounds including their hydrolysis or de-glycosylation.

Overall, so far no concrete dietary feed additive or functional ingredient can be suggested for pig diets to reduce enteric CH_4 formation in pigs in practice. There is still a need for studies with standardized products or extracts. For plant metabolites, plant extracts and methane inhibitors showing potential effects, data obtained in *in vitro* studies must be confirmed *in vivo*. The consequences of such interventions on the growth performance, animal health, environmental impact and sustainability have to be evaluated in an integrative way. Future research with aim to control and mitigate enteric methane production in pig farming could be envisaged taking into account these factors.

5 Interventions to reduce CH_4 emission from pig manure

The quantity and composition of faeces as well as the urea content and pH of the urine of pigs are related to the composition and intake of diets by pigs and to the extent to which nutrient supply is matching with the nutrient requirement of the pig. These factors also largely determine the methane and ammonia formation capacity of pig manure after excretion in the pen and during storage. Consequently, feeding management, ingredient and nutrient composition and the use of additives have significant effects on the chemical and bacterial composition of the manure as mixture of faeces and urine. This chapter aims to review nutritional interventions that could mitigate CH_4 emission from pig houses and pig manure.

5.1 Emission of methane from pig manure

5.1.1 Calculation of CH_4 emission from manure

Methane emission from manure is caused by the degradation of organic matter in manure under anaerobic conditions. CH_4 originates from the succession of microbial processes (Hellmann et al., 1997; Monteny et al., 2006). Initially, bacteria convert easily degradable substrates into VFAs, CO_2 and H_2. This extensive microbial activity increases the temperature of the manure and provides suitable conditions for methanogenic archaea to convert acetate, CO_2 and H_2 into methane under a thermophilic and anaerobic environment. Overall, factors that favour CH_4 formation in manure are lack of oxygen, high temperature, high moisture content, a high concentration of degradable organic matter, a neutral pH, a low redox potential, and a C/N ratio of between 15 and 30 (review of Philippe and Nicks, 2015).

According to the IPPC guidelines for National Greenhouse Gas Inventories[25] (Dong et al., 2006), CH_4 emissions from animal manure can be calculated as follows :

$CH_4 = VS \times B_0 \times MCF$, in m^3 , based on the:
- amount of excreted volatile solids (VS) in manure[26] in kg;
_ biochemical CH_4 potential (B_0[27]) also known as maximum methane-producing capacity of the manure, in m^3 CH_4 / kg VS;
_ methane conversion factor (MCF), in %, that reflects the portion of B_0 that can be converted into CH_4 in real conditions of each type of manure management system. It can be calculated from measurements in storage simulation trials in laboratory or *in vivo* experiments in pig houses.

IPCC (Dong et al., 2006) has published values for VS, B_0 and MCF for different regions of the world, different climates, livestock categories and manure storage systems. For Western or Eastern Europe, the recommended value for VS is 0.30 kg[28] /pig /day for fattening pigs (Dong et al., 2006). In the Netherlands, the Tier 2 calculation using the National Emission Model Agriculture (NEMA) based on year 2021 published the excretion value of 117 kg VS/animal/year for fattening pigs (van Bruggen et al, 2023[29]). For gilts, sows, young boars and breeding boars, these values are 140, 341, 140 and 198 kg, respectively.

The measurement of B_0 can be performed *in vitro* using bio-methanogen potential (BMP) tests (Dong et al., 2006). Jarret et al. (2011b) have described a standard method adapted from Vedrenne et al. (2008). Briefly, the manure samples are anaerobically incubated for 16 days at 38 °C in bottles with an inoculum.

[25] The methods, tier 2 and tier 3, from IPCC (2006) use country specific information on composition and management of manure.
[26] Volatile Solids is a measure of the organic matter content of wastewater or manure that can be lost under specific heating conditions. (Hamilton and Zhang, 2016)
[27] also referred to as biochemical methane potential (BMP) or Biochemisch methaanpotentieel (BMP)
[28] Default estimates are ±20%
[29] Appendix 28 of NEMA report

During incubation, biogas formation is regularly monitored by pressure measurement of the headspace of the bottle and the methane content in biogas is analysed. The average B_0 value proposed by IPCC (Dong et al., 2006) with Tier 1 approach is 0.45 m^3 per kg VS. B_0 values in literature vary from 0.29 to 0.53 m^3 CH_4 /kg VS (Møller et al., 2004a; Chae et al., 2008; Vedrenne et al., 2008; Jarret et al., 2011b; Dämmgen et al., 2012). For the Dutch conditions, Groenestein et al. (2016) calculated B_0 of 0.22, 0.31 and 0.34 m^3 CH_4 /kg VS for cattle, pig and poultry manure, respectively. Calculations for 1990-2021 with NEMA gave the excretion value of 0.31 m^3 CH_4/kg VS (van Bruggen et al, 2023 [30]).

MCF values range from 2% to 80% in the literature according to manure type, manure management, storage duration, diet composition and temperature (Møller et al., 2004a; Jarret et al., 2011b; Dämmgen et al., 2012; Rodhe et al., 2012). The values proposed by Dong et al. (2006) for pig manure are 13.7% for solid storage, 2.8% for pit storage < 1 month and 69.8% for pit storage > 1 month. It was shown that during long-term storage (90 days), the MCF of pig slurry value increased from 5.3 to 31.3% at temperatures ranging from 15 to 20 °C, respectively (Møller et al., 2004a). The latter authors also found that, at 20 °C, reducing the storage duration to 30 days decreased the MCF to 2.8%. A maximal MCF of 72% was established by Zeeman (1994) for pig manure stored 180 days at 30°C. Groenestein et al. (2016) calculated a mean MCF of 36% for manure of pigs and sows in Dutch conditions. Interestingly, the type of diet has a rather limited effect on B_0 value, according to Jarret et al. (2011b). They found that the effect of diet was much more marked on MCF. MCF is also highly affected by storage time and management of manure.

The degradation of the organic matter in liquid manure is a complex biological process. Certain fermentation processes can conflict with each other. Interestingly, several authors (Hashimoto, 1986; Angelidaki and Ahring, 1994; Chynoweth et al., 1998; Hansen et al., 1998, Vedrenne et al., 2008) indicated that potential inhibiting compounds (total VFA and free NH_3) could limit the anaerobic digestion of manure during the hydrolytic or methanogenic phases. According to Vedrenne et al. (2008), even if the total VFA concentration seems to be involved in the inhibition of methanogenesis, other parameters such as the propionate : acetate ratio, are known to modulate methanogenesis in manure. However, the results of Vedrenne et al. (2008) indicated that NH_3 was probably not the compound responsible of the inhibition of methanogenesis. No correlation was found between both parameters and the concentrations of free NH_3 in the manure samples showing the lowest methane formation (10-304 mg NH_3/L) were largely below inhibiting thresholds for free NH_3 (850 and 1335 mg/L, respectively for cattle and swine manure), found by Angelidaki and Ahring (1994) and Hansen et al. (1998).

5.1.2 CH_4 emission from pig houses under practical conditions

In pig houses under practical conditions, CH_4 emissions consist of two parts, release from manure and direct formation of enteric gas by animals. Emissions may be very different within each physiological stage of pigs, as reviewed by Philippe and Nicks (2015). In addition to climatic conditions, the bedded systems (fully or partly slatted floor, litter systems), the manure or litter removal strategy and the storage duration inside the building appear to play an important role (Vellinga, 2023). For all categories of pig or sow manure, higher emissions are observed with a longer duration of indoor manure storage. A system that daily removed the manure out of the barn resulted in a reduction by 90% of methane emission from the house of fattening pigs in the study reported by Booijen et al. (2023).

Petersen et al. (2016) used 11 cattle and 20 pig manure samples collected beneath slatted floors on six Danish farms to estimate in laboratory assay total methane emissions of 0.011 kg and 0.030 kg CH_4/kg VS[31] from cattle and pig manure, respectively, assuming a retention time in pits of 15 and 30 d for pig and cattle manure, respectively. This significantly lower CH_4 production rate observed with cattle manure was partly explained by the lower storage temperature in cattle houses with passive ventilation, but degradability of VS in cattle excreta was probably also lower, according to Petersen et al (2016).

Regarding manure, most of the CH_4 emission is produced during storage under anaerobic conditions and high temperature (Møller et al, 2004b; Sommer et al, 2007) whereas little emission follows land application (Montes et al., 2013). Manure produces less CH_4 when handled as a solid (e.g., in stacks or pits) or when deposited on pasture (USDA, 2016).

[30] Table 5.1 of NEMA report
[31] 1 kg CH_4 = 1.49 m^3 CH_4 at atmospheric pressure and 15° C

Hence, authors indicated that main opportunities to reduce CH_4 emission are centred on preventing anaerobic conditions during manure storage or capturing and transforming the CH_4 that is produced, if anaerobic conditions are present (Montes et al., 2013; Vellinga, 2023). Data summarized by Chianese et al. (2009) indicated average CH_4 emissions from covered slurry, uncovered slurry, and stacked manure to be 6.5, 5.4, and 2.3 kg/m^2 per year although rates vary with temperature and time of storage.

A natural crust is naturally formed during the storage of unprocessed manure, whereas no crust is formed with processed manure in which solids are reduced (Rotz et al., 2015). Farmers aim to limit manure crusting which interferes with efficient pumping and land application and could allow development of a fly population. When no crust is formed, no N_2O emission from liquid manure is assumed (Dong et al., 2006). However, NH_3 emissions are also affected by a hard naturally formed crust, which prevents ammonia produced by manure from escaping (Smith et al., 2007). In addition, manure crusting may also reduce methane emission as methane-oxidising bacteria in the hard crust can oxidise methane into CO_2 (Petersen and Ambus, 2006). It was also demonstrated that artificial crusting with straw cover reduced CH_4 emission from swine manure stores (Laguë et al., 2005). Accordingly, a semi-porous organic or inorganic material capable of supporting micro-organisms can encourage the growth of CH_4 oxidizing microbes (Nielsen et al, 2013)

Calculating the relative share of enteric formation and manure emission of CH_4 may be difficult and imprecise. Particularly, we have not found *in vivo* studies in pigs measuring or calculating the total volumes of CH_4 from enteric formation on the one hand, and emission from manure on the other. As a consequence, emissions for pigs in The Netherlands are usually calculated with CH_4 emission factors from enteric fermentation based on Tier 1 default values of IPPC (van Bruggen et al., 2020).

The average emission factor for gas released from swine manure proposed by IPPC (Dong et al., 2006) for Western Europe including inside and outside storage is 32.9 g CH_4/ head /day. Taking into account the daily enteric CH_4 emissions proposed for fattening pigs and reproductive sows, i.e 2.4-2.5 and 6.0-8.0 g CH_4 /head proposed by Vermorel et al. (2008) and Dämmgen et al. (2012), it can be estimated that 7.5 to 20% of the total CH_4 pig farm emission is related to enteric methane formation.

5.1.3 Microbial communities in the GIT and in manure

The microbial population in the manure has a crucial role in the CH_4 emission dynamics. As a consequence, research has investigated about management aspects influencing microbial population and therefore CH_4 emission during storage inside the pig houses. Literature indicated that the easily degradable organic matter contributes to CH_4 emission from manure as a result of anaerobic environment. As a consequence, adding large amounts of easily digestible OM promote the growth of acidogenic microorganisms resulting in reduced pH. As example, addition of a glucose-rich substrate (brewing sugar) in manure influenced microbial anaerobic respiration, resulting in a reduction of livestock manure pH to <5.0, through self-acidification caused by lactic acid production (Bastami et al, 2016). Subsequently, CH_4 emissions were significantly reduced by 87 and 99% in the cool (10°C) and warm (30°C) environments. In the same study reported by Bastami et al. (2016), a microorganism treatment reduced CH_4 emissions by 17 and 27% in the cool and warm environments, respectively.

However, few studies have investigated the composition of the methanogenic archaea community in both intestinal digesta or faeces, and that in manure of pigs. As example, analyses of the microbial community in the swine manure were undertaken by Pepple et al. (2012), Kumar et al. (2020) and Ramesh et al. (2021) but these studies did neither study methanogenic species nor the relationship between the microbiome in the GIT and diet ingredient or nutrient composition. Yet, Seradj et al. (2018) found no difference of the methanogen structure between the middle colon digesta and the fresh manure in the pit. In their study, a higher dietary CP content increased counts of total bacteria and total methanogen archaea in the intestine as well as in the manure. They underlined that the similar structure may be due to the ability of the methanogen archaea species to adapt efficiently to the new environment in the manure.

A high abundance of methane producing microbes has been found in pig manure, as shown by Prenafeta-Boldú et al. (2017) with the molecular quantification of the archaeobacteria domain (around 10^7 gene copy numbers/ml). From PCR amplification of archaeal 16 rDNA in a swine manure storage pit, Whitehead and Cotta (1999) identified groups of sequences similar to *Methanobrevibacter* sp., *Methanocorpusculum* sp., and *Methanoculleus* sp.

In the pig manure of two Danish farms, the characterization of methanogens by T-RFLP and qPCR analysis targeting mcrA by Petersen et al. (2014) revealed one prominent T-RFs fingerprint associated with *Methanoculleus* spp. In one of the farms, *Methanosarcinales* spp., as well as *Thermoplasmata*-related methanogens were identified.

Prenafeta-Boldú et al. (2017) also showed a strong shift from the hydrogenotrophic genus *Methanobrevibacter*, predominant in the fresh pig manure, towards the obligate aceticlastic *Methanosaeta* at the end of the biochemical methane potential assays (36 d) following IPCC standards (Dong et al., 2006). They underlined that this may have resulted from adding an external inoculum collected from a mesophilic anaerobic digester of a pig manure facility, to the biochemical tests, as *Methanosaeta* spp. are found ubiquitously in anaerobic digesters. However, the replacement of *Methanobrevibacter* spp. by other archaeal species such as *Methanosarcina* and *Methanoculleus* has been demonstrated to occur spontaneously in swine manure storage tanks (Peu et al., 2006; Barret et al., 2013). After 40 d of storage of pig manure, Shin et al. (2019) also identified that the majority of the archaeal community was assigned to *Methanosarcina* (70.4%), *Methanolobus* (8.8%), *Methanobrevibacter* (7.9%), *Methanocorpusculum* (5.4%), *Methanomassiliicoccus* (1.2%), and *Methanobacterium* (1.1%). For the eubacteria domain, the main microbial groups identified by Prenafeta-Boldú et al. (2017), as Bacteroidetes, Bacilli and Clostridia, contained most of the known fermentative groups of bacteria in pig manure and in anaerobic digesters.

So far, characterization of the archaeal domain in pig manure is mainly based on investigations of DNA sequences, and few methanogen species have been isolated and cultivated. This makes precise taxonomic grouping difficult. However, it appears that the microbiome of the manure may be substantially influenced by the microbiome in the digestive tract of pigs and possibly by diet composition. Therefore, reducing the methane and ammonia forming capacity of the manure through manipulation of the manure microbiota may be appropriate. Although there is a lack of research results on this topic, dietary strategies may also influence the relative composition and density of methanogens in both the GIT and in manure of pigs.

5.2 Dietary mitigation of CH_4 emission from pig manure

In pig production, reducing dietary protein or increasing dietary fibre are efficient ways, already used in practice, to reduce NH_3 emissions. These strategies by changing the ingredient composition may directly or indirectly affect CH_4 emissions. Literature has shown that reducing dietary protein decreased the potential formation of CH_4, whereas this potential is increased when dietary crude fibre increased.

5.2.1 Influence of dietary fibre on CH_4 emission from manure

Previous research has addressed the impact of dietary fibre on CH_4 and other GHG emissions. At laboratory scale, most studies reported higher CH_4 emissions from manure when diets with a higher fibre level are fed, resulting in more fermentable organic matter in the faeces and manure. CH_4 emissions have been shown to increase by 32 to 76% from the manure samples collected by Velthof et al. (2005), and Jarret et al. (2012) from fattening pigs fed with 13 to 25% NSP, and 11 to 14% NDF, respectively (Table 7). Velthof et al. (2005) studied how the combination of different fibre sources in the feed could influence methane emission after 90 days of storage. The latter authors found that emission of CH_4 increased with an increasing concentration of total NSP in the diet. Emission of CH_4 was lowest for the manure derived from the diet with lowest content of NSP. The low emission was attributed to low total C and VFA concentrations in the manure. Results of Velthof et al (2005) showed that, for a same quantity of total NSP, a higher dietary DNSP content through inclusion of SBP, did not result in an increase in methane emission from manure. Possibly, SBP was fermented in the pig's digestive tract and excreted only slightly in faeces. However, comparing pigs fed with 0 to 20% SPB diets, Clark et al. (2005) found significant difference in CH_4 formation in exhausted air from manure storage vessels for 17% CP diets but not with 14% CP diets (Table 7). Lastly, in the study reported by Philippe et al (2015), the inclusion of SBP in replacement of wheat increased both NSP and DNSP dietary contents resulting in significant higher emission in room air (Table 7). Seradj et al. (2018) showed that manure from animals fed high fibre diet presented higher abundances (Log N° copy/g fresh matter) of total bacteria (9.7 vs. 9.5), total archaea (9.2 vs. 8.8) and total methanogenic archaea (6.6 vs. 6.4) than from those receiving a low fibre diet.

Table 7 *Effects of dietary fibre content on CH4 emissions measured in pig houses or in vitro from manure.*

Reference	Fibre ingredients	Total NSP	Dig NSP	NDF	CH_4 emission	*P*	Animal	System
		g/kg	g/kg	g/kg				
Velthof et al., 2005	1 vs 12% OH, 7 vs 5% SBP, 5 vs 7.5% WB, 5 vs 5% MGF	129 /189	83 /83	nr	3.3 vs 6.1 g C/kg	nr	finishers	*in vitro*
	5 vs 16% OH, 10 vs 10% WB, 5 vs 0% MGF, 0 vs 8% Ac	129 /245	62 /62	nr	4.4 vs 5.8 g C/kg	nr	finishers	*in vitro*
	0 vs 16% OH, 16 vs 8% SBP, 0 vs 10% WB, 0 vs 6% MGF	129 /245	104 /104	nr	1.8 vs 5.0 g C/kg	nr	finishers	*in vitro*
	5 vs 0% OH, 0 vs 16% SBP, 10 vs 0% WB, 5 vs 0% MGF	129 /129	62 /104	nr	4.4 vs 1.8 g C/kg	nr	finishers	*in vitro*
	16 vs 16% OH, 0 vs 8% SBP, 10 vs 10% WB, 0 vs 6% MGF, 8 vs 0% Ac	245 /245	62 /104	nr	5.8 vs 5.0 g C/kg	nr	finishers	*in vitro*
Clark et al., 2005	0 vs 20% SBP (17% CP)	nr	nr	nr	2.37 vs 2.43 log10 µL/L	*	growers	storage vessel
	0 vs 20% SBP (14% CP)	nr	nr	nr	2.42 vs 2.38 log10 µL/L	NS	growers	storage vessel
Jarret et al., 2012	0 vs 15% DDGS	nr	nr	111 /143	55 vs 97 L/d/pig	***	growers	*in vitro*
Jarret et al., 2011a	0 vs 20% DDGS vs 20% SBP vs 20% RSM	105 /125 /221 /151	nr	105 /155	70 vs 120 vs 95 vs 128 L/d/pig	***	growers	*in vitro*
Trabue & Kerr, 2014	0 vs 35% DDGS	nr	nr	75 /131	18.5 vs 21.9 g/d/AU	NS	late finishers	storage tanks
Li et al., 2011	0 vs 20% DDGS	nr	nr	144 /163	21.0 vs 33.3 g/d/AU	**	fatteners	room air
Pepple, 2011	0 vs 22% DDGS	nr	nr	nr	84 vs 60 g/d/AU	nr	wean to finish	barn air[1]
Philippe et al., 2015	0 vs 37% SBP	201 /440	109 /302	198 /300	21.0 vs 41.5 g/d/AU	***	gestating sows	room air
	0 vs 23% SBP	190 /314	85 /195	178 /234	27.2 vs 37.9 g/d/AU	***	fatteners	room air

nr : not reported, AU: animal unit= 500 kg BW, *P:* Statistical significance: * P<0.05: *, P<0.01**, *** P<0.001, NS: P>0.05. OH: oats hulls, WB: wheat bran, MGF: maize gluten feed, SBP: sugar beet pulp, DDGS: dried distiller grains with solubles, RSM: rapeseed meal. [1]: from two different farms

At animal house level, emissions originate from the release by animals (enteric formation) and from the manure stored under the slatted floors of pens. When the experimental design provides for frequent emptying of the manure, concentrations measured in the air in the room correspond almost entirely to enteric formation, as in the studies reported by Seradj et al. (2018) and Booijen et al. (2023). Fibrous diets with up to 48% NSP provided to fattening pigs or gestating sows housed on slatted floors or bedded floors have been reviewed by Philippe and Nicks (2015) and were shown to increase CH_4 emission by 13-52% based on the concentration of methane measured in pig rooms, compared to using basal diets (18-26% NSP).

Jarret et al. (2012) compared CH_4 emission from manure of fattening pigs fed a conventional diet (11% NDF) based on cereals and SBM or a fibrous diet (14% NDF) with 15% RSM and 15% dried distiller's grain with solubles (DDGS). They found higher emissions (55 vs 97 L CH_4 /pig) with the fibrous diet during a 100 d storage simulation of manure. Interestingly, the ultimate CH_4 potential (B_0) was not affected by the type of diet when expressed as L CH_4 / kg VS but was significantly affected when expressed as L CH_4 /pig /day, in agreement with previous results from Jarret et al. (2011a) and the methodology of calculation of Dong et al. (2006). This result was the consequence of a higher quantity of OM excreted via the faeces when feeding fibre-rich diets, related to a lower nutrient digestibility of such diets. Indeed, the fibrous diet significantly increased the amount of faeces excreted by 40%, whereas urine excretion was not affected. Concurrently, the fibrous diet significantly increased the C excretion by 51% and the amount of OM excreted per pig by 65%, compared to the control diet. In this regard, the effluents from the fibrous diet produced 60% to 66% more CH_4 per pig and per day compared to the control diet. These results are in agreement with the previously mentioned swine feeding trials with DDGS diets showing a significant increase in CH_4 emission for pigs fed DDGS diets compared with control diets (Li et al., 2011; Jarret et al., 2011a,b).

Besides that, a diet containing 35% DDGS supplied to late fattening pigs by Trabue and Kerr (2014) resulted in reduced manure pH, increased surface crust coverage, increased manure DM content and increased manure C, N and S contents compared to a corn-soybean meal diet. For pigs fed DDGS diet compared to corn-SBM fed pigs, the CH_4 concentration in the manure (5.35 vs 5.24 µL/L, respectively) and the calculated emission factor of CH_4 from stored manure (21.9 vs 18.5 g CH_4/day/pig, respectively) were numerically but not significantly increased. Furthermore, concentrations in air over the manure and emission factors per pig for NH_3 and H_2S were significantly lower in animals fed DDGS.

Trabue and Kerr (2014) pointed out that one factor that most likely affected CH_4 emission between diets was the larger surface crusting of manure from animals fed DDGS diets compared with corn-SBM diets. Surface crusting of manure has been shown to reduce CH_4 emission. High concentrations of NH_3 and H_2S (Petersen et al., 2012; Sutaryo et al., 2013) may also partially explain why there was no significant difference between manure samples with regard to CH_4 emission in the study of Trabue and Kerr (2014).

In the study of Seradj et al. (2018) with dietary CP and fibre level as studied factors in three-phase experimental diets for weaning-growing pigs[32], the fibre-rich diets increased CH_4 emission in the animal room (mean 5.0 vs 4.0 g CH_4 /d/pig for high and low fibre contents, respectively). CH_4 emission from the manure pits was not altered by the dietary fibre level in phase 1 and 2 but was significantly affected in the final phase (9.7 vs 14.8 g CH_4 /m^3 manure for high and low fibre contents, respectively).

Similarly, Pepple (2011) found no significant differences of CH_4 concentration of a wean-to-finish pig farm using 22% DDGS (48 ± 35 g/d-pig of CH_4) or a farm using a traditional corn-soybean ration (72 ± 65 g/d-pig) in deep-pit facilities. The author underlined that there was considerable seasonal variation in H_2S and CH_4 emission.

Lastly, because more fat may be included in the diets to reach the same net energy content, fat content of faeces could be higher for the fibrous diets as in the study reported by Jarret et al. (2012). According to the latter authors, this may contribute to higher CH_4 formation for the fibrous diet. Indeed, it was also shown by Li et al. (2002) that the addition of different fat sources to cow or pig manure increases CH_4 formation during anaerobic co-digestion in a fermenter.

Furthermore, Philippe and Nicks (2015) underlined that, overall, cumulative GHG emissions (sum of CO_2, CH_4 and N_2O) seem to be little influenced by the presence of dietary fibre. This can be seen in previous studies by the same group of authors regarding emissions of pigs receiving 37 to 42% SBP. At house level, CH_4 formation was increased but total emissions calculated as CO_2 equivalent ranged from -6 to +9% compared with emissions produced by pigs given a conventional diet (Philippe et al., 2009, 2012a,b).

In conclusion, most of the studies showed that increasing the dietary fibre content resulted in a higher methane emission from pig houses and from manure. It also seems that the impact of the proportion of dietary digestible fibre on CH_4 emission from manure depends on the total amount of fibre and on the digestive fermentation capacity of the pig. Some results showed that increasing the proportion of digestible NSP in a constant quantity of total NSP does not lead to an increase in CH_4 emission from manure. Other results indicated that if quantities of total and digestible NSP are increased and exceed the fermentative capacity of the animal, the excreted fibre can result in methane emission from the manure. Lastly, the effects of the quantity of excreted organic matter, storage duration and temperature, and crust coverage are very significant, independent of the biochemical CH_4 potential factor (B_0).

[32] In the study of Seradj et al. 2018, CP contents were 19.8 vs 17.2%, 17.3 vs 15.2% and 17.5 vs 12.5% for phase I (6-11 weeks of age), II (12-16 w) and III (17-21 w), respectively and NDF contents were 141-154 vs 120-123, 174-162 vs 130-126 and 175-167 vs 123-135 g/kg, for phases I to III, in a 2x2 design.

5.2.2 Influence of dietary protein content on CH_4 emission from manure

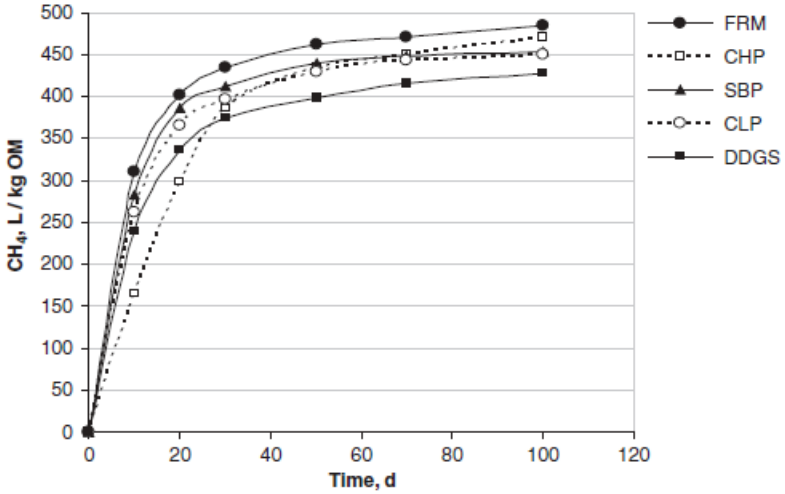
Whereas dietary interventions to reduce the nutrient excretion have been studied for many years, especially for N and P (Van der Peet-Schwering et al., 1999; Dourmad and Jondreville, 2007), its consideration as a way to reduce CH_4 and N_2O emission from manure is relatively new, according to Montes et al. (2013). Actually, it has been established in the past decades that diets reduced in crude protein content but supplemented with free amino acids improve the efficiency of protein utilization, and reduce N excretion via urine and subsequent NH_3 emission (Mroz et al., 1993; Canh et al., 1997 and 1998; Portejoie et al., 2004; Aarnink and Verstegen, 2007). Literature also reported that dietary protein reduction may result in an increase, reduction or non-significant differences of the enteric CH_4 formation (See 4.1.2), whereas only a few studies have been carried out for the emission of methane from the manure under varying CP content.

Measuring CH_4 emissions from manure samples in the laboratory, Velthof et al. (2005) reported a reduction by 21% (4.8 vs 6.1 g C/ kg manure) as result of a lower dietary crude protein (14.2% vs 18%) for fattening pigs. One reason could be the lower VFA content measured in manure produced from the low protein diet as during anaerobic manure storage, VFA can be transformed into CH_4 (Zeeman, 1994).

Interestingly, this reduction was simultaneous with the decreased emission of NH_3 during manure storage and lower N_2O emission from soil-applied manure. Accordingly, CH_4 and NH_3 emissions in the house, but not N_2O emissions, were reduced with CP reduction in the study of Cappelaere et al. (2023) who compared diets with a CP content of 18.1, 16.9 and 15.0% in phase 1 (28-48 kg) and 16.1, 15.0 and 13.8% in phase 2 (48-80 kg). Interestingly, methane emissions were higher with the control treatment in phase 1 (1.09, 0.68 and 0.71 g CH_4/ kg body weight gain, respectively), and similar among treatments in phase 2 (0.96, 0.95 and 1.04 g CH_4/kg gain, respectively). These differences were mainly explained by the differences of daily gain in phase 1 (946, 1127 and 1161 g/day, respectively) and phase 2 (1116, 1271 and 1138 g/day, respectively) whereas feed intake was similar among treatments.

Other results did not corroborate these findings. No significant effect was observed of a reduced crude protein level (13.6% vs 15.9% CP, respectively) on CH_4 emission (4.1 vs 3.6 g CH_4 /d/pig for low-protein and control diets, respectively) in the house of finishing pigs, whereas NH_3 emission and manure pH were lowered by dietary protein reduction in the study reported by Hansen et al. (2014). Laboratory-scale experiments based on samples of manure also reported non-significant differences (Le et al., 2009, 15.0 vs 12.0% CP; Osada et al., 2011, 17 vs 14.5% CP) or increases (Clark et al., 2005; 16.8 vs 13.9% CP) in CH_4 emission of fattening pigs fed reduced protein diets. Atakora et al. (2004) also calculated that CH_4 emission from pig manure was identical with low or high protein diets, commensurate with the C excretion. In the above-mentioned study of Seradj et al. (2018), the dietary protein level had no significant effect on total CH_4 emission in the animal room (mean 4.9 and 4.1 g CH_4 /d/pig for high and low protein contents, respectively) and, accordingly, on CH_4 emission from the manure pits. Furthermore, in the same study, no interaction occurred between the dietary crude protein and fibre contents on CH_4 emission.

Emission kinetics over time could explain some of the differences in these studies. For instance, Jarret et al. (2011a) reported that the diet with a 14 % CP content reduced the CH_4 B_0 of growing pigs by 5% compared to the control 17.5% CP diet. These authors reported that, the formation of CH_4 over the first 20 days, was the lowest for the 17.5% CP treatment, whereas after 100 days of incubation it was the highest, indicating that CH_4 formation from the manure of animals fed high protein diets may occur later (See Figure 17).



CHP control high protein growing diet (17.5% CP, 2.2% CF); CLP control low protein diet (14% CP, 2.1% CF); DDGS dry distiller's grain with solubles diet (18.4% CP, 3.5% CF); SBP sugar beet pulp diet (17.6% CP, 5.6% CF); FRM fatty rapeseed meal diet (17.7% CP, 4.5% CF). Reproduced from: Jarret et al., 2011a.

Figure 17 *Effects of dietary ingredient and nutrient composition on cumulative formation of CH4 (B0).*

As conclusion, optimizing the animal's diet to improve N efficiency, balancing dietary N input with production level, and limiting excretion of fibre while reducing enteric CH_4 fermentation, are important steps in reducing N_2O and CH_4 emissions from manure. Therefore, due to numerous interactions on gas emission at the animal, storage, and land application phases, GHG mitigation practices should not be evaluated as separate factors but as a component of the whole livestock production chain (Montes et al., 2013).

5.2.3 Influence of low-impact and circular diets on CH_4 emission from manure

Traditional feed formulation combines ingredients into diets that simultaneously meet animal nutrient requirements and minimise cost, but ignore some indirect environmental impacts (Garcia-Launay et al., 2018). New approaches have optimised the environmental impacts of low-impact pig diets by including new constraints into a multi-formulation method, e.g. animal excretion in P and N, climate change, eutrophication, demand in non-renewable energy, acidification or land occupation at both feed mill gate and farm gate (Mackenzie et al., 2016; Garcia-Launay et al., 2018; De Quelen et al., 2021).

Currently, the concept of circularity is proposed to address key sustainability issues related to animal production (Puente-Rodriguez et al., 2022). Local or alternative feed ingredients (e.g. plant based protein sources, food waste, insects, or seaweed) are evaluated on the basis of circularity principles: protect ecosystems, use and recycle biomass, limit food–feed competition, fairness, animal health and welfare (Puente-Rodriguez et al., 2022). Feeding pigs with low-opportunity-cost feed such as agricultural residues and by-products, and better use of local feed resources are discussed as strategies for a transition towards more circular animal feeds (Stroosnijder et al., 2022; Gatto et al., 2024).

A few studies have evaluated the impact of circular or low-impact feeds on the composition and ammonia emission and methane formation from manure. Low-impact feeds differ from conventional feeds because of a higher proportion of co-products and EU-cropped protein rich ingredients, resulting in a higher fibre content (Garcia-Launay et al., 2018; De Quelen et al., 2021). The higher dietary fibre content may affect the manure composition and associated gaseous emissions (Portejoie et al., 2002; Jarret et al., 2010). In the study of De Quelen et al. (2023), the quantities of DM, OM, C and N excreted /pig /day were increased for pigs fed low-impact diets based on co-products and legume seeds, as result of their lower nutrient digestibility. The authors found that the biochemical CH_4 potential (B_0) differed only to a small extent between the two batches of manure. However, when this potential was expressed per pig per day, a higher methane production potential was observed for the low-impact diet in agreement with the difference in OM excreted via the faeces, as previously found by Jarret et al. (2010).

5.2.4 Effects of dietary mitigation measures on other GHG emissions from manure

Some mitigation measures regarding methane formation may have opposed effects on the formation of ammonia and other GHG.

The main conflicting effects are expected from the inclusion of fibrous ingredients in the diet. Previous research has shown that the amount of organic matter that can be fermented in the colon (i.e. digestible NSP) is a substrate for enteric methanogenesis by archaea, whereas the impact of fibre in faeces and manure on CH_4 emission from the manure is more unclear. Besides that, the inclusion of fibrous feed ingredients in the diet can lead to a reduction of NH_3, in relation with less urinary nitrogen excreted as reviewed by Van der Peet-Schwering et al. (1999). Interestingly, the content of digestible NSP in pig diets is accordingly used as a practical indicator for a lower urinary N-excretion by the Chamber of Agriculture of Lower Saxony in Germany (Meyer, 2020).

Fibrous feed ingredients may lead to a reduction of the urea concentration in the urine. Non-starch polysaccharides in the diet induces a supply of potentially fermentable organic matter to the colon microbiome inducing microbial growth and a flux of urea from the blood to the lumen of the large intestine. The urea is broken down to NH_3 by bacterial urease and used for microbial protein synthesis. This protein synthesis causes less NH_3 to be reabsorbed from the colon, resulting in the shift of some of the nitrogen excretion from urine to faeces (Mroz et al., 1993; Kirchgessner et al., 1994, Bakker, 1996; Canh et al., 1997; Jarret et al., 2011a). Therefore, no effect was found of dietary NSP on the total N excretion, but the partitioning of N excretion between urine and faeces differed between diets in the study reported by Canh et al. (1997). The pigs fed the diets with the highest NSP content excreted less nitrogen in the urine and more in the faeces resulting in a decrease of NH_3 emission.

Table 8 *Effects of a reduction in dietary crude protein content on emissions of CH_4, CO_2, N_2O and CO_2 measured in pig houses or from manure obtained from pits.*

Reference	Diet CPC	CH_4	CO_2	N_2O	NH_3	Animals	Context
Atakora et al., 2011ab	16.2 vs 19.5%	-19%	-6%			fatteners	resp. chambers
Atakora et al., 2011ab	16.0 vs 19.5%	-24%	-6%			fatteners	resp. chambers
Atakora et al., 2011ab	16.2 vs 19.0%	-6%	-6%			fatteners	resp. chambers
Cappelaere et al., 2023	15.0 vs 18.1%	-35%		-11%	-58%	growers	resp. chambers
Cappelaere et al., 2023	13.8 vs 16.1%	8%		26%	-12%	finishers	resp. chambers
Clark et al., 2005	13.9 vs 16.8%	10%	10%	NS		fatteners	manure vessels
Velthof et al., 2005	14.2 vs 18.0%	-21%			-54%	fatteners	manure in vitro
Le et al., 2009	12.0 vs 15.0%	-32%	14%	5%	-29%	fatteners	manure pits
Seradj et al., 2018	12.6 vs 17.5%	-11%			0%	piglets	manure pits
Philippe et al., 2006	14.4 vs 17.6%	-13%	3%	96%	-26%	fatteners	room air
Hansen et al., 2014	13.6 vs 15.9%	15%	10%			growers	room air
Seradj et al., 2018	12.6 vs 17.5%	-16%			-40%	piglets	room air

Methane (CH_4), carbon dioxide (CO_2), nitrous oxide (N_2O) and ammonia (NH_3). Differences calculated as significant by authors are underlined.

The main effect of organic matter fermentation is that N assimilated in organic form via bacterial protein into the large intestine and excreted in the faeces is less rapidly degraded to NH_3 than urea which is rapidly converted into NH_3. In the study of Jarret et al. (2011a), the faecal N excretion was twice higher (10 vs 5 g N/pig per day) for three high-fibre diets including 20% of DDGS, SBP or RSM compared to the control diet based on wheat and SBM. Furthermore, dietary fermentable NSP lead to formation of VFAs in the hindgut and in the manure of pigs which decreases the pH of the manure (Aarnink et al., 1994; Canh et al., 1997, 1998; Mroz et al., 2000; Jarret et al., 2011a). The pH is also strongly related with NH_3 emission, with lower pH levels decreasing NH_3 emissions. Accordingly, research in Denmark, France and The Netherlands showed that the pH and NH_3 emission from the manure were reduced when the level of SBP in the diet of growing pigs was increased, as reviewed by Van der Peet-Schwering et al. (1999).

In agreement with these results, including biofuel co-products, rich in fibre, to pig diets in the study reported by Jarret et al. (2011a) resulted in a decrease by 19% to 33% in the emission of NH_3 from manure during storage and an increase by 73%, 37% and 84% in the amount of CH_4 emitted from manure per pig.

The effects of the reduction in crude protein content of pig diets on the emissions of CH_4, CO_2, N_2O and CO_2 in air in pig houses or above manure pits are presented in Table 8. For several studies, a decrease both in CH_4 and NH_3 concentrations were measured (Velthof et al., 2005; Philippe et al., 2006; Le et al., 2009; Seradj et al., 2018; Cappelaere et al., 2023). However, Philippe and Nicks (2015) recalculated that the cumulative house emissions of GHG (i.e. sum of CO_2, CH_4 and N_2O in CO_2 equivalents[33]) were increased by 7% in their previous work (Philippe et al., 2006) with pigs on litter fed with a diet reduced in crude protein content (18.5 vs 17.5% and 15.5 vs 14.0% CP for the growing and finishing periods respectively). These authors underlined that despite lower CH_4 emission (-13%, 15.0 vs 13.1 g/d), this was due to a higher contribution of N_2O from the litter (+96%; 0.52 vs 1.02 g/d).

In conclusion, CH_4 mitigation measures should be applied in such a way to avoid increased NH_3 emissions, or "pollution swapping" as underlined by Montes et al. (2013). Dietary strategies to mitigate ammonia and GHG emissions in pigs should be considered in an integrated way considering enteric and in animal house formation as well as during storage of manure.

5.3 Effect of feed additives on CH_4 emission from pig manure

Several feed additives or specific feed ingredients potentially interesting for reducing enteric CH_4 formation by animals may also have beneficial effects on GHG emissions, including CH_4 and NH_3, from manure. A few studies on feed supplementation with additives give some information on the concentration of VFA and on GHG formation in manure.

Enzymes such as cellulases and hemi-cellulases supplemented to animal diets to counteract anti-nutritional effects of fermentable fibres could have a further lowering effect on enteric CH_4 formation and could possibly reduce methane emission from manure. Even though, β-glucanase and β-xylanase supplementation increased ammonia emission from manure of pigs fed a barley-based diet but not when providing an oat-based diet in the study of O'Shea et al. (2010). However, for both barley- and oat-based diets, the acetate : propionate ratio in manure was increased by enzyme supplementation which could indicate more methanogenesis in manure. The addition of xylanase to high-protein diets caused a decrease in CO_2 and CH_4 emissions from manure, but an increase when added to low-protein diets in the study reported by Clark et al. (2005). They indicated that this might be due to differences in the NSP composition of the high and low-protein diets. The supplementation of phytase has been shown to increase feed efficiency and protein deposition in pigs, possibly also leading to a decrease in ammonia and GHG emissions by pigs (Ball and Möhn, 2003), but in their review, Philippe and Nicks (2015) failed to find studies on the effects of the dietary addition of phytase on GHG emissions by animals and from manure. In addition, Yitbarek et al. (2017) found no effects of supplementing swine diets with phytase on methane emission from soil after manure application.

Using *Yucca Schidigera* extracts in diets has been proposed as a means to inhibit gut urease activity, to modulate selected microorganisms, and to bind gastrointestinal and faecal NH_3 (Duffy and Brooks, 1998) According to the authors, *Y. schidigera* is predominantly used as an agent controlling NH_3 formation. For example, Amon et al. (1995) measured a reduction in NH_3 concentration in pig buildings with the addition of *Yucca shidigera* extract to the feed and manure. However, the effects of the inclusion of Yucca extract in pig diets on CH_4 and N_2O emissions from pig houses are still unknown according to Philippe and Nicks (2015). In a study reported by Colina et al. (2001), the addition of *Yucca schidigera* extract to the diet of nursery pigs reduced NH_3 concentrations in air of the nursery rooms, but had no effect on manure NH_3 concentration and manure pH. A mixture of essential oils and saponins (Fresta® F Plus) that supposedly reduce the effect of urease in the manure was added in the diet of finishing pigs by Holm (2010). The supplementation did not result in significant differences in NH_3 emission, nor on pH of the manure.

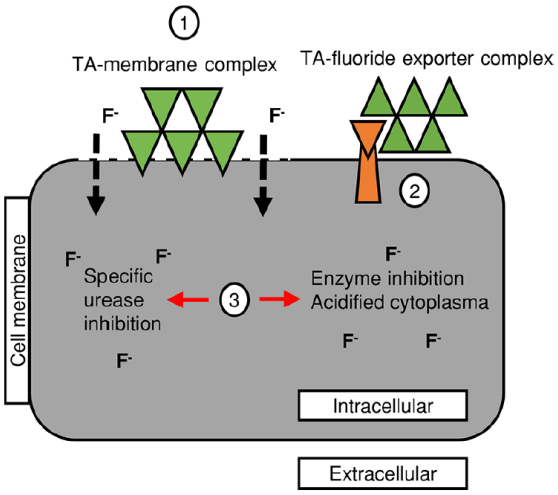
[33] On the basis of a global warming potential of 25 and 298 CO_2equiv. for CH4 and N2O, respectively.

Besides that, the *Bacillus* spp. supplementation added by Prenafeta-Boldú et al. (2017) to high fibre pig diets (with 20% DDGS and 20% wheat middling's) reduced both methane and NH_3 potential volatilization from pig manure by >40% and 50%, respectively, on fresh weight basis, compared to the control diet. An apparent dose-related negative effect was consistently observed upon dietary *Bacillus* spp. supplementation on the amount of organic matter (volatile solids, total carbon and content of chemical oxygen demand) in the dejections. Treatment diets also resulted in a reduction of total nitrogen and ammonia whereas VFA content of manure tended to be higher. However, no clear differences among dietary treatments appeared for the composition of the eubacteria and archaeal domains in fresh and digested pig manure. The effect of inclusion of *Bacillus* spp. in the diet on the microbial composition of manure was not significant.

Maigaard et al. (2022) investigated in dairy cows whether dietary fat, nitrate and 3-NOP that reduce enteric CH_4 formation in rumen would also decrease CH_4 emission from manure. They concluded that each of the interventions reduced enteric methane without affecting CH_4 emission from manure, with the exception for additional fat supplementation for which methane yield from manure was increased by 18%.

Recently, Dalby et al. (2020) reported the discovery of a microbial inhibitor combination consisting of tannic acid and sodium fluoride (TANaF), which exhibited synergistic inhibition of *in vitro* NH_3 production when supplemented in pure bacteria culture and in pig manure, while simultaneously inhibiting CH_4 and odorant (H_2S and VOC) emissions. Microbial community analysis and gas emission data suggested that TA-NaF acts as an efficient generic microbial inhibitor, and Dalby et al. (2020) hypothesized that the synergistic inhibitory effect on ammonia formation is related to tannic acid damaging the cell membrane of micro-organisms allowing intracellular fluoride ions to inhibit urease activity of ureolytic bacteria (Figure 18).

It has been known for several decades that acidifying manure in the pit can lead to a reduction in NH_3 emission (Hendriks and Vrielink, 1996; Fangueiro et al., 2015). Moreover, acidification could also be an efficient way to reduce CH_4 emissions from pig manure. In a study with two Danish farms (Petersen et al., 2014), the acidification in-house (pH 5.5) or in-store (pH 6.5) of the manure resulted in reduction of cumulative CH_4 emission by 99 and 94% during the storage period.



Tannic acid (TA) binds to the cell membrane enabling unhindered fluoride ion (F^-) passage (1). Tannic acid binds to fluoride exporter proteins or related surface components, leaving them dysfunctional (2). Intracellular fluoride ion concentration rises due to the disruption of cross membrane gradient, inhibiting urease and enzymes central to metabolism and acidifying the cytoplasma (3). Tannic acid molecules are shown as green triangles. Membrane bound fluoride exporter is coloured orange. Reproduced from: Dalby et al., 2020.

Figure 18 *Suggested synergistic effects and inhibition mechanisms of tannic acid and sodium fluoride on bacterial cell walls and cell metabolism.*

The dominance of *Methanosarcina* was reduced as the pH of manure was decreased (67% vs 46% and 34% abundance for pH 8.4 [non-acidified], 6.0 and 5.0, respectively) after 40 d of storage in the study of Shin et al. (2019). However, compared to the non-acidified manure (10.6 L CH_4/L), the acidified pig manures showed higher biochemical CH_4 potential (12.7–14.6 L CH_4/L), presumably as result of the storage of degradable organic matter in manure under acidic conditions. A CH_4 emission of 3.7 kg CO_2 eq./ton manure during 40 d of storage was measured from the non-acidified manure, which was reduced to 1.8 kg and 0.1 kg CO_2 eq./ton at pH 7.0 and pH 5.0, respectively.

However, the maximum reduction was achieved at pH 6.0 and the drop was not substantial from pH 6.0. Yet, it appears also that kinetics of the CH_4 reduction could reflect the adaptation of the bacteria to manure acidification according to Philippe and Nicks (2015).

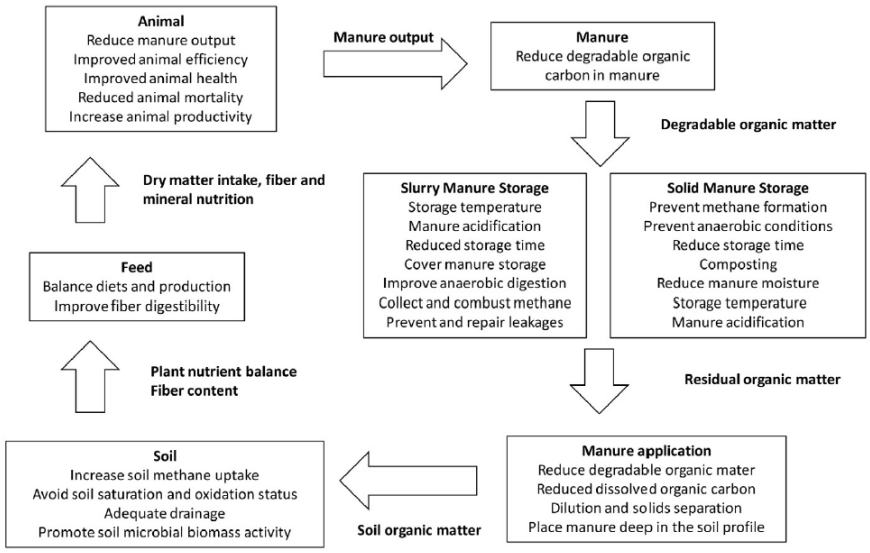
The pH of the manure can also be reduced by lowering the pH of the urine. In agreement with previous results (Mroz et al., 1997; Canh et al., 1998), den Brok et al. (1999) showed that the addition of a mixture of organic acids, mainly benzoic acid, to the diet of growing-finishing pigs may resulted in lower pH of urine (by 7.8 to 2.5 units), of manure (0.5 to 0.8 units) and lower NH_3 emission. Benzoic acid has been reported to reduce the urinary pH of pigs in a dose dependent manner (Kluge et al. 2010) and to decrease ammonia emission from pig manure (Kristensen et al., 2009 ; Eriksen et al., 2010). This reduction in manure pH, together with the toxic effect of sulphides or the direct impact of benzoic acid could result in inhibition of methanogenic archaea. However, results are less clear on the effects of diet acidification on CH_4 emission from manure. Whereas a previous report of Eriksen et al. (2010) showed a transient inhibition of CH_4 emissions during storage of manure from pigs fed a diet with 2% benzoic acid, a following study (Eriksen et al., 2014) indicated, relative to the control treatment, no effect of 1% benzoic acid. Similarly, it was shown by Aarnink et al. (2008) that 1% addition of benzoic acid to the diet of growing finishing pigs lowered the pH of the urine (5.29 and 6.50 for acid and control treatments, respectively) and significantly reduced NH_3 emission by 16%. However, the inclusion had no effect on CH_4 emission (27.5 and 28.1 g/d per pig for control and benzoic acid treatment, respectively).

Overall, there is a lack of information about effects of individual feed additives on the CH_4 emission from manure. Whereas direct acidification of the manure in the pit has shown to reduce manure CH_4 emission, results are inconsistent about effects of supplementation of the diet with acids. Some promising results are reported with probiotics and with a combination of tannic acid and sodium fluoride (TANaf) but with the latter one, only *in vitro* results are available. Feeding studies using extracts of *Y. Shidigera* or 3-NOP have not investigated the CH_4 emission from pig manure. Lastly, studies evaluating effects of dietary enzymes have reported no effects on CH_4 emission from manure. To conclude, the claim that feed additives that improve nutrient digestibility and performance in pigs could potentially reduce CH_4 and other GHG has rarely been tested and validated experimentally under field conditions.

5.4 Combined approaches for reduction of CH_4 emission by pigs

5.4.1 Effects of manure management system on CH_4 emission

Different animal and manure management practices are currently used or proposed to effectively reduce CH_4 NH_3 and N_2O emissions during manure storage and/or land application. For pigs, the major part of CH_4 is produced during manure storage. Consequently, the most efficient way to obtain a substantial reduction of CH_4 emissions from pig manure is the implementation of alternative techniques to standard manure management methods, i.e. storage in deep pits underneath the housing system and then in an outdoor pit. These techniques concern the frequency of manure removal, reduction of the storage time, lowering of manure temperature by storing it outside during colder seasons, and may be complemented by dietary strategies (Montes et al., 2013, Figure 19).



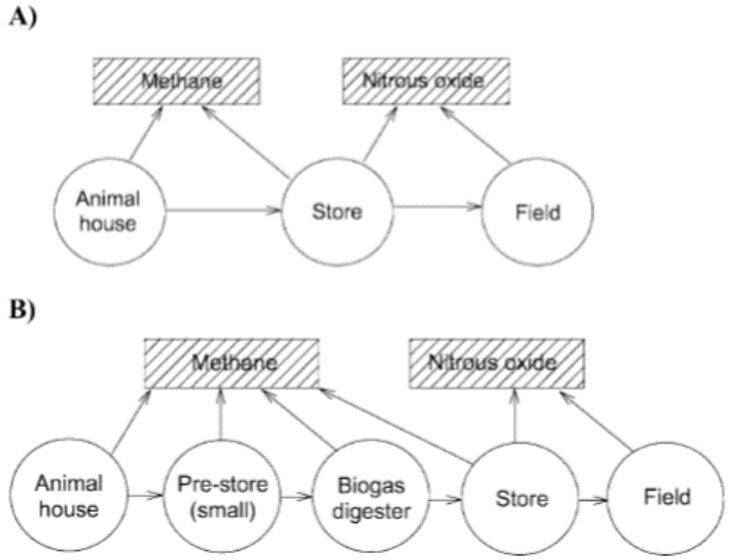
Reproduced from: Montes et al., 2013.

Figure 19 *Flow of organic carbon through the livestock production system and opportunities to mitigate CH4 emission.*

Moreover, it has been shown that manure separation can contribute to reduce CH_4 emission from slurry (mixture of faeces and urine) by producing liquid fraction low in DM and OM. VanderZaag et al. (2018) measured after solid-liquid separation (SLS) a 81% reduction of CH_4 emission on a per-L basis, on average, compared to raw manure. The mean ultimate CH_4 emission potential (B_0) per kg of volatile solids (VS) was 247±8 L CH_4 /kg VS for raw manure and 221±9 L CH_4 /kg VS for separated liquid (-11%). Besides that, the speed of CH_4 production was increased by SLS, due to removing a large fraction of lignocellulose from the liquid fraction to the solid fraction, leading to relatively high CH_4 production rates per unit of time from the remaining OM with a higher relative content of easily degradable dissolved components. According to VanderZaag et al. (2018), to considering both the degradation rate and total potential (B_0) is essential when evaluating these techniques, because actual emission reductions will vary depending on the storage conditions.

Dalby et al (2021) reviewed how methane production can be reduced by manipulating key variables through management procedures of animal manure. The authors also highlighted that a detailed understanding is necessary for developing accurate models for calculating CH_4 emission from liquid manure, with particular focus on the microbiological conversion of organic matter to CH_4.

It should be taken into account that anaerobic digestion by a digester together with energy-rich co-digestates to produce biogas from animal effluents is also very effective in reducing CH_4 emission and organic C content of manure (Sommer et al., 2004; Levasseur and Quéral, 2022).



Reproduced from: Sommer et al., 2004.

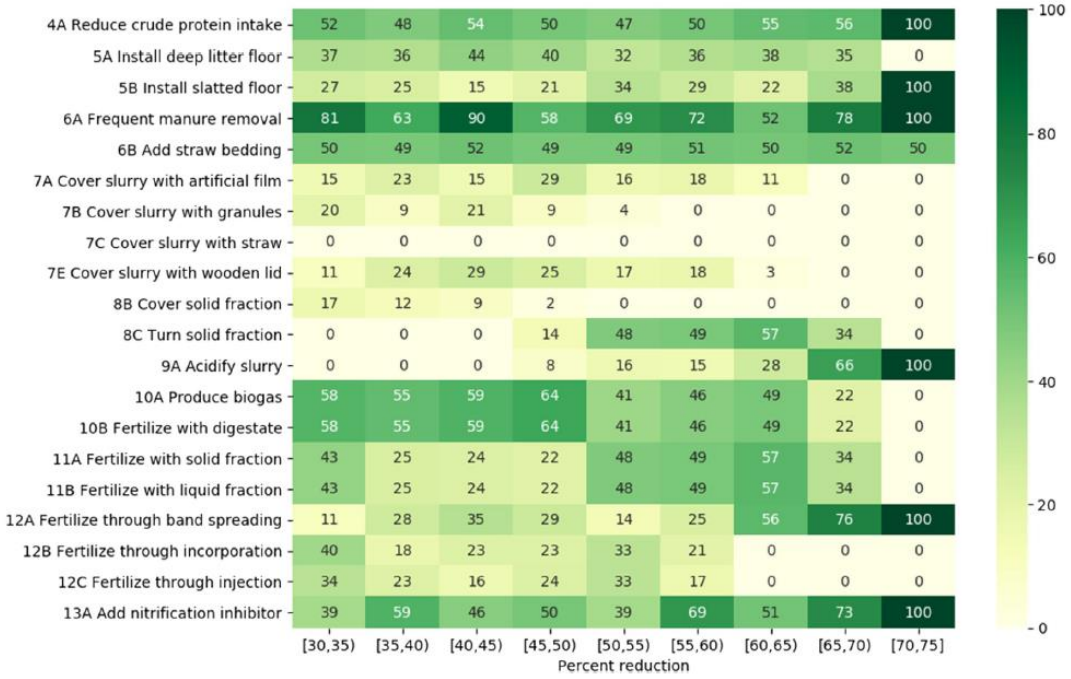
Figure 20 *Sources of CH4 and N2O during in-house and outside storage and field application of manure using A) the traditional management system or B) an anaerobic digester for animal manure.*

Such manure management system using a digester to produce energy highly impacts the economical and environmental concerns of methane formation on farm (Figure 20). In Denmark it was aimed that 50% of the total animal manure volume should be fermented on farm or in centralised biogas digesters (Petersen et al., 2016).

5.4.2 Effects of combined mitigation approaches on CH_4 emission

Puente-Rodríguez and Groenestein (2019) have made an inventory of technical measures which are able to reduce the methane emissions of the Dutch livestock production systems (i.e. cattle and pig farming): animal breeding, composition and quality of feed, use of inhibitors, frequent manure removal from barns, manure additives, manure mixing and aeration, manure cooling, anaerobic digestion, and methane oxidation. Authors developed a qualitative assessment framework on the basis of literature and expert consultation to evaluate different sustainability and ethical aspects of each measure from the perspective of different stakeholders (e.g. farmers and citizens and considering the environment, animal welfare, autonomy and fairness).

Recently, aan den Toorn et al. (2021) have evaluated the potential of combined approaches for reduction of CH_4 and N_2O emissions in the meat and dairy supply chains from three emissions sources: enteric fermentation, manure management, and fertilizer application. These authors have identified 44 mitigations measures from literature and identified possible combinations using mathematical graphs. Finally, they calculated the emission reduction of each combination to assess mitigation effects on total combined CH_4 and N_2O emissions expressed in CO_2-eq compared to a baseline scenario. Regarding the composition of the diet, measures included the reduction of the crude protein content and supplementation of diets with additives, but a decrease in dietary fibre content was not considered as measure. They found that the combinations with the highest mitigation reduced the CH_4 and N_2O emissions of pigs by 70%. Overall, the combinations with a high mitigation potential showed a pattern of a few core mitigation measures providing the largest contribution to reducing total emissions, combined with a wider set of other measures (Figure 21). The number of measures in a combination appeared to influence how much of the CH_4 and N_2O emissions can be mitigated.



Reproduced from: *aan den Toorn et al., 2021.*

Figure 21 *Heatmap showing for each mitigation measure applicable for pigs the share of combinations in which it is included (colour and value), with the combinations grouped by the percent reduction of the total combined CH4 and N2O emissions in CO2-eq compared to the baseline.*

Essential measures were either measures with particularly low impact factors compared to competing measures, or measures that do not compete with others. Good examples for the former are '9A Acidify slurry' for swine. The measure '13A Add nitrification inhibitor' is also an example of a measure that is compatible with all others although it does not have a strong impact. Moreover, the essential measures partially depend on which factor to total emission is relatively large for the particular livestock such as CH_4 from enteric fermentation for ruminants, and CH_4 from manure management for swine (aan den Toorn et al., 2021).

However, few studies have experimentally verified *in vivo* if some combined mitigation measures selected *in silico* could reduce effectively the emission. As example, synergistic effects of the combination of tannic acid and sodium fluoride on both NH_3 and CH_4 have been reported in the study of Dalby et al. (2020). Consequently, the impacts of the different combinations require more experimental research to verify the combined emission reduction potential. As highlighted by aan den Toorn et al. (2021), future experimental research should focus on potential addition of mitigation measures which could have high impact on the total combined CH_4 and N_2O emissions in CO_2-eq.

5.5 Conclusion

The integration of approaches that could mitigate both CH_4 formation from pig GIT and emissions from manure is an important potential solution for consideration. Mechanisms of methanogenesis are similar for both biological systems, and fermentable organic matter, i.e. digestible NSP or volatile solids, is the substrate of the degradation in anaerobic conditions by synergistic bacteria and archaea. However, it is difficult to determine with precision the potential impact of a change in the composition of the diet on the total CH_4 formation from both the pig house and the manure, because of the current lack of references. A higher fibre content of the diet, for instance resulting from using more circular and low-impact feed ingredients, is related to a high content in digestible NSP and to a lower digestibility of nutrients leading to higher excretion of organic matter fermentable in the manure. Because of this, CH_4 formation would likely be higher from both the GIT and the manure. There is no precise information, however, on the relative contribution of organic matter in a pig diet that can be fermented in the digestive tract to form CH_4, and the proportion that cannot be fermented in the GIT but can be later fermented in the manure to form CH_4.

Furthermore, the dietary fibre content interacts with other parameters affecting anaerobic fermentation and CH_4 emission from manure, such as manure crusting and kinetics of gas emission.

Overall, it seems important to improve the efficiency of utilization of nutrients and reduce the amount of N and C excreted by the pig via the faeces and urine relative to their intake via the diet, and to limit the formation of methane and other GHGs in the manure. As example, reducing the concentration of protein in the diet and using free amino acids could be an important measure in the mitigation of methane formation.

Although a few results are reported from *in vitro* studies, there is a general lack of information about effects of feed additives on the CH_4 emission from pig manure. There is a need for more research to validate experimentally the potential ability of feed additives to reduce CH_4 and other GHG under practical conditions. Furthermore, although very few studies have investigated the community of the archaeal domain in the GIT and in the manure, the relationship between these microbial communities should be more systematically investigated in future research on the influence of dietary strategies aiming to reduce total CH_4 formation.

Finally, dietary mitigation interventions cannot be addressed separately from other management measures related to housing of animals and storage of manure. Suggestions were made that implementing combined measures in pig husbandry could create opportunities for further reduction of CH_4 formation and emission. For all approaches, research should be carried out to investigate the effects of dietary mitigation measures on total emissions of CH_4 and other GHG, via both enteric formation and emission from manure.

Conclusions

This desk study reviews literature on the effects of nutrition of pigs on methane formation in the gastro-intestinal tract and on methane emission from manure. The main findings are displayed in Appendix 3.

The fermentation of organic matter in the large intestine and, after excretion, in the manure, is an important and unavoidable aspect of the intake and digestion of diets by pigs. Methane (CH_4) is formed by specific microorganisms of the archaeal domain. The formation is the result of the activity of a complex microbial ecosystem requiring the synergistic contribution of several microorganisms under anaerobic conditions.

Three metabolic pathways of methanogenesis coexist, the hydrogenotrophic, acetogenic and the methylotrophic pathway. The hydrogenotrophic pathway, using CO_2 and H_2 as substrates, is the most common and is performed by most of the methanogenic archaea in the colon. Methanobacteriaceae is the main archaea family in the colon of pigs, and hydrogenotrophic *Methanobrevibacter* spp. and methylotrophic *Methanosphaera* spp. are the dominant species of the microbiome in the GIT of pigs. *Methanobrevibacter* spp. are more efficient for CH_4 formation. Acetate and propionate concentrations in colon, as end products of fermentation, are significant predictors of methane formation, as CH_4 formation is correlated positively with the concentration of acetate and the acetate : propionate ratio, and negatively with the propionate concentration.

Some CH_4 mitigation strategies could be based on increasing the diversity of the methanogenic community and the relative abundance of *Methanosphaera* spp., on adapting the competition for H_2 and CO_2 utilization between reductive acetogens and methanogens, as well as on increasing the concentration of propionate relative to acetate. However, a large number of archaea species involved in methanogenesis are not yet fully identified. The acquisition of methanogenic archaea by the host is largely unknown but animal breed, age, dietary and environmental conditions play a role. At present, only a few studies on dietary factors influencing the enteric CH_4 formation have investigated the archaeal population present in the hindgut of pigs. This should be a key element of future research.

In the gastro-intestinal tract, CH_4 formation increases from the proximal to the distal part of the large intestine and CH_4 is predominantly released in the air via flatulence. For measuring *in vivo* the enteric CH_4 formation, use of respiration chambers and measurement of methane in in- and outgoing air is the standard method but recent alternative approaches or methods using sensors or NIR analysis of faeces have been developed for ruminants and could be adapted to use in pigs.

Average enteric methane formation reported by literature is 0.8, 2.5 and 6-8 g CH_4 per animal per day for piglets, fattening pigs and adult sows, respectively. The enteric formation of CH_4 in pigs depends mainly on the age and body weight of the animal, the feed intake and the chemical and nutritional composition of the diet, specifically the soluble fibre content. The digestible NSP appears as the best indicator of the amount of organic matter fermented in the colon by the microbiome. Equations have been proposed to estimate the enteric CH_4 formation by pigs or adult sows from the daily intake of digestible NSP. Consequently, future *in vivo* or *in vitro* studies assessing the effects of diet composition on the CH_4 formation should consider the fraction of digestible NSP.

Research has shown that decreasing the dietary NSP content can reduce enteric CH_4 formation. Reducing the crude protein content or increasing the fat content could mitigate the enteric gas formation as well. However, little detailed information is available on how the composition of the diet and fermentation capacity of the animal influence the CH_4 formation by pigs. Interactions between fibre, protein and fat concentrations in the diet and the role of the host microbiota are poorly documented, and only limited information is available on the effects of feeding strategies to mitigate CH_4 formation in commercial farming conditions.

The literature suggests that some specific feed additives such as saponins, tannins, plant extracts and essential oils, constituents of algae and methane inhibitors (e.g. 3-NOP, organic acids) could mitigate methanogenesis in the rumen of ruminants.

However, limited information is available in non-ruminants, and *in vitro* research as well as *in vivo* investigations should be undertaken to explore their efficacy under farming conditions.

Total CH_4 emission from pig houses consists of enteric formation by animals and release from manure during the in-house and outdoor storage. Depending of the animal housing and manure management conditions, enteric methane represents 8 to 20% of the total CH_4 pig farm emission. Uncontrolled fermentation in manure during collection and storage results in formation of methane that is emitted into the environment, while controlled anaerobic fermentation of organic matter in manure in digesters results in methane used as biogas. The methane potential of manure is affected by diet composition. A higher CH_4 release from the manure may result from using low-impact, circular and fibre-rich diets, having a lower nutrient digestibility resulting in higher excretion of fermentable organic matter. However, still little information is available on the impact of the composition of the diet on total CH_4 formation and release from both pig house (enteric methane and methane formed and released in the pen and manure pit underneath the floor) and during manure storage and application outside the barn. Furthermore, in spite of interesting results from *in vitro* studies, information about effects of feed additives on the CH_4 emission from pig manure is lacking. In addition, only a few studies have investigated the community of archaea responsible for methane formation in the GIT and in the manure of pigs and their relationship.

Only a few studies have explored the effects of dietary mitigation measures on both enteric formation and manure emission of CH_4. In addition, the other GHGs produced at digestive or manure level (i.e. CO_2, NH_3, N_2O, H_2, H_2S) should be more systematically studied as well in studies focussing on methane as primary target. Lastly, dietary mitigation measures to reduce methane emission by pigs cannot be separated from other management measures related to animal housing and manure storage. Research using modelling approaches indicates that combining measures could create opportunities for further reduction in CH_4 formation and emission in pig production.

Overall, there is a need for more research to experimentally validate the potential ability of diet composition and feeding strategies to reduce emission of CH_4, other GHG and ammonia under practical conditions.

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Appendix 1 Methanogens identified in digesta and faeces/excreta in different non ruminant animal species

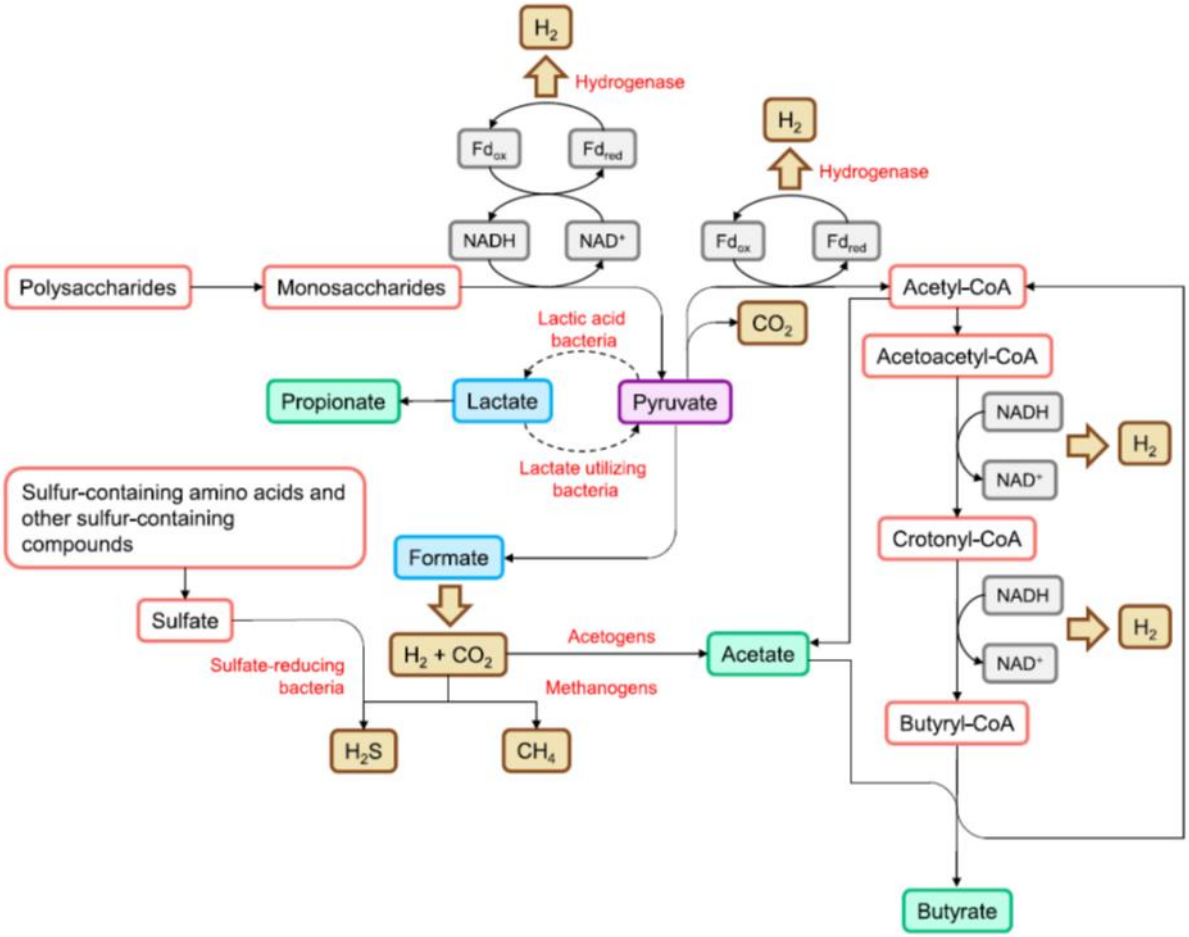
Animal	Taxon-related methanogen[1]	Reference
Swine Colon	**Methanobacteriales**: *Methanobrevibacter [Mbb. boviskoreani* (99.7%), *Mbb. gottschalkii* (98.3%), *Mbb. millerae* (98.7%), *Mbb. olleyae* (98.7%), *Mbb. ruminantium* (98.9%), *Mbb. smithii* (99.7%), *Mbb. wolinii* (96.1%)], *Methanosphaera* [*M. stadtmanae* (88%)]; **Methanomicrobiales**: *Methanoculleus spp*. (100%) **Methanomassiliicoccales**: *Methanomassiliicoccus* [*M. luminyensis* (88.2%)]	Luo et al., 2013 Luo et al., 2017 Mi et al., 2019
Faeces	**Methanobacteriales**: *Methanobrevibacter [Mbb. boviskoreani* (100%), *Mbb. gottschalkii* (98.8%), *Mbb. millerae* (98.5%), *Mbb. olleyae* (>97%), *Mbb. ruminantium* (98.8%), *Mbb. smithii* (99.9%), *Mbb. thaueri* ((>97%)], *Methanosphaera* [*M. stadtmanae* (97.2%), *M. cuniculi* (98.1%)]	Mao et al., 2011 Luo et al., 2012 Su et al., 2014 Federici et al., 2015
Horses Hindgut	**Methanobacteriales**: *Methanobrevibacter spp*. (100%); **Methanomicrobiales***: Methanocorpusculum spp*. (100%); **Methanosarcinales**: *Methanimicrococcus* [*M. blatticola* (99%)]; **Methanomassiliicoccales**: *Methanomassiliicoccus spp*. (100%)	Murru et al., 2018
Faeces	**Methanobacteriales**: *Methanobrevibacter [Mbb. arboriphilus* (>97%), *Mbb. gottschalkii* (100%), *Mbb. ruminantium* (98%), *Mbb. smithii* (97%)], *Methanobacterium spp*. (>97%), Methanosphera spp. (>97%); **Methanomicrobiales**: *Methanocorpusculum* [*M. labreanum* (96%), *M. parvum* (>97%)]; **Methanosarcinales**: *Methanimicrococcus [M. blatticola* (94%)]; **Methanomassiliicoccales** (97%)	Yamano et al., 2008 Fernandes et al., 2014 Lwin and Matsui, 2014
Donkeys Hindgut	**Methanobacteriales**: *Methanobrevibacter spp*. (100%); **Methanomicrobiales**: *Methanocorpusculum spp*. (99%), *Methanomicrobium [M. mobile* (100%)]; **Methanomassiliicoccales**: *Methanomassiliicoccus spp*. (100%)	Murru et al., 2018
Rabbits Caecum	**Methanobacteriales**: *Methanobrevibacter* [*Mbb. arboriphilus* (99.6%), *Mbb. gottschalkii* (99.2%), Mbb. smithii (99.9%), Mbb. woesei (99.5%)], *Methanobacterium spp.* (>97%), *Methanosphaera* (>97%) [*M. cuniculi (c. w.)]*, *Methanothermobacter spp*. (>97%)	Biavati et al., 1988 Kušar and Avguštin, 2010 Liu et al., 2018
Chickens Caeca	**Methanobacteriales**: *Methanobrevibacter (Mbb. woesei)*; **Methanomicrobia** (p. n. i.); **Thermoplasmata** (p. n. i.); **Methanococci** (p. n. i.); **Methanopyri** (p. n. i.)	Saengkerdsub et al., 2007a Qu et al., 2008
Feces	**Methanobacteriales** (p. n. i.); **Methanomicrobiales**: *Methanogenium spp. ((c. w.))*	Miller et al., 1986 Saengkerdsub et al., 2007b
Geese Feces	**Methanobacteriales**: *Methanobrevibacter (Mbb. woesei (c.w.))*	Miller et al., 1986 Miller and Lin, 2002

1 The closest known methanogen based on the percentage of similarity of 16S rDNA or 16S rRNA gene sequence (p. n. i. – percentage not indicated); or methanogen detected based on cell wall analysis (c. w.)

Adapted from Misiukiewicz et al., 2021.

Appendix 2 Biochemical pathways of intestinal gas production by monogastrics

Reproduced from Mutuyemungu et al., 2023.



Biochemical pathway of H_2, H_2S, CH_4, and CO_2 formation from microbial fermentation. Gases are shown in brown boxes; intermediate products of fermentation are shown in blue boxes and primary products of fermentation (SCFA) are shown in green boxes.

Appendix 3 Main results of the literature study

Topic and information	Paragraph
Enteric methane formation by methanogenic microbiota	
The Methanobacteriaceae archea family is the dominant methanogen in colonic digesta of pigs accounting for approximately 71% of identified methanogens. Analysis of archaeal diversity in swine colonic digesta showed the presence of *Methanobrevibacter* spp., *Methanosphaera* spp., Methanomassiliicoccales and Methanomicrobiales.	2.1.2
Whereas *Methanobrevibacte*r spp. are the most abundant of archaea identified in pig faeces, (46% of clones), unidentified archaea species make up 55% of clones.	2.1.2
Methanogens have an extreme genetic diversity, but they can utilize only a limited number of substrates: carbon dioxide (CO_2), acetate and compounds containing methyl groups.	2.3
Most organic compounds such as carbohydrates, volatile fatty acids (VFA) and alcohols are not direct substrates for methanogens and have to be fermented first by syntrophic bacteria, protozoa or fungi to acetate, formate, H_2 and CO_2, before their use by methanogens.	2.3
Hydrogenotrophic pathway of CH_4 is the most common and is performed by the majority of the methanogens. Methylotrophic pathway is at second rank and aceticlastic pathway at third	2.3.1
Methanosphaera spp. produce smaller amounts of CH_4 than *Methanobrevibacter* spp.	2.3.1
Microbiota ecology	
Differences in methanogenesis between individuals are estimated smaller in pigs than in other species consuming less standardized diets.	2.4.1
Archaeal composition in the hindgut is dynamic and is affected by age and breed of pigs, but environmental parameters may also play a crucial role.	2.4.1
Diversity and activity of methanogens in the digestive tract can be influenced by the diet.	
Oxidation-reduction potential (Eh) in the digestive tract is an important factor of the growth of methanogens. Hindgut of finishing pigs had a stricter anaerobic environment than rumen.	2.4.1
A lean Landrace pig was shown to have a greater diversity and higher numbers of methanogen genes in faeces than a fat Erhualian breed pig.	2.4.2
There is a relationship between the energy balance of a pig and the microbial utilization of dietary polysaccharides with involvement of archaea.	2.4.2
Fermentation processes in hindgut of pigs	
In the hindgut (caecum and colon) of pigs, dietary fibre is degraded by the microbiome at variable extents, depending of nature of carbohydrates and degree of lignification.	2.1.1
The hindgut fermentation results in formation of short chain fatty acids (SCFA), CO_2, H_2, CH_4, urea and heat.	3
Five gases: N_2, 0_2, $C0_2$, H_2 and CH_4 constitute more than 99% of the total gas formed in the GIT of pigs.	2.1.1
Small amounts of CH_4 are found in gas from caecum, followed by a steady increase in colon, reaching concentrations as high as 29 to 37% in rectum. The colon is the site showing the highest CH_4-forming activity.	3.1.1
Limited quantitative information is available on the routes of excretion of the intestinal produced CH_4 in pigs. It can be speculated that a predominant part is excreted via flatulence.	3.1.2
Quantitative enteric CH_4 formation in pigs	
Older and heavier pigs have higher ability to ferment the fibre fraction of the diet as result of larger microbiome in the gut, increased capacity of intestinal microbiome to ferment fibre, increased transit time and lower relative feeding level.	3.3.1
CH_4 formation is more than 2.5 higher for sows with a body weight of 210 kg than for fattening pigs in the weight range 60–115 kg.	3.3.1
Calculated relative to feed intake and using diets with equal fibre contents, CH_4 formation by kg of DM intake is about 1.7 higher for pigs of 150 kg than for pigs of 50 kg.	
High fibre diets lead to a higher rate of non-enzymatically digested and non-absorbed substrates that reach the colon.	3.3.2
Methanogenic activity is stronger related to the content of soluble fibres.	3.3.2
High soluble fibre diets exhibit a lower apparent ileal digestibility of non-starch polysaccharides (NSP) and total carbohydrates and a higher total tract digestibility of NSP and total carbohydrates, and a lower faecal excretion of energy compared to other diets.	3.3.2
Reduced feed intake allows more time for fermentation in GIT.	3.3.3

Compared to growing pigs, adult sows have a higher fermentative capacity of diets with high amounts of insoluble fibre. The difference in fermentation capacity is smaller for diets high in soluble fibre.	3.3.4
Calculation of enteric CH_4 formation	
Tier 1 and 2 methods of IPCC do not take into account the influence of diet composition on enteric CH_4 formation. Hence, this approach is a poor estimate of enteric CH_4 formation.	3.4.1
The DNSP fraction is the best indicator of the amount of organic matter fermented in the colon by the microbiome.	3.4.1
The same definition of the organic matter fermented in the large intestine is published in Denmark, France, Germany and the Netherlands but using different names (Digestible NSP, Digestible Residue, Fermentable Fibre or BFS).	3.4.1
Digestibility of fat in the small intestine is very high, resulting in absence of dietary fat in the hindgut.	3.3.3
Dietary mitigation of enteric CH_4 formation	
Inclusion of feed ingredients rich in soluble (digestible) NSP such as sugar beet pulp and other pectin containing ingredients should be limited in pig diets to reduce enteric CH_4 formation.	4.1.1
Reduction in CH_4 formation resulting from decrease of dietary CP content may be a side effect of a lower fibre content of diet.	4.1.2
Little information is available on effects of dietary lipid content on enteric CH_4 formation.	4.1.3
Feed processing of pig diets increase nutrient digestibility and may reduce feed intake and enteric CH_4 formation.	4.1.4
Dietary supplementation with *Saccharomyces cerevisiae* could reduce pig enteric CH_4 formation.	4.2.2
Bacteria utilizing hydrogen (e.g. acetogens) may compete with methanogenic archaea for H_2.	4.2.2
Biologically active plant metabolites (tannins, saponins and essential oils), and algae have been extensively studied as strategies to reduce enteric CH_4 formation in ruminants, and modes of action may have a potential in non-ruminants. However, no specific information is available on effects of saponins or algae on enteric CH_4 in pigs.	4.2.3-4.2.4
3-Nitrooxypropanol (3-NOP) acts on the enzyme responsible for CH_4 formation, and is one of most promising solutions for mitigation of CH_4 emission by dairy cows. No studies have been undertaken on use of this additive in pig diets for reducing enteric CH_4 formation in hindgut.	4.2.5
Little *in vivo* or *in vitro* data has been published on effects of inclusion of organic acids in the diet enteric CH_4 formation in pigs.	4.2.5
Future genetic selection in pigs may include new traits related to modulation of enteric CH_4 formation.	4.3
Dietary mitigation of manure CH_4 emission	
Thermophilic and anaerobic environment in manure provides suitable conditions for methanogenesis.	5.1.1
The type of diet may have a limited effect on the biochemical CH_4 potential (B_0 value), i.e. the maximum CH_4-producing capacity of the manure, and more marked on the methane conversion factor (MCF), that reflects the portion of B_0 that is converted into CH_4 in real conditions.	5.1.1
Free NH_3 in manure is probably not responsible of inhibition of methanogenesis.	5.1.1
It can be estimated that 8 to 20% of the total CH_4 pig farm emission is related to enteric methane formation.	5.1.2
Although few other results are available, one study showed no difference of the methanogenic archaea community between the middle colon digesta and the fresh manure.	5.1.3
The replacement of *Methanobrevibacter* spp. by species as *Methanosarcina* and *Methanoculleus* occurs spontaneously in swine manure storage tanks.	5.1.3
Increasing the dietary fibre content results in higher CH_4 emission from pig from manure.	5.2.1
If quantities of total and digestible NSP are increased and exceed the fermentative capacity of the animal, the excreted fibre can result in CH_4 emission from manure.	5.2.1
Circular or low-impact diets mays result in higher CH_4 potential in agreement with the difference in OM excreted via the faeces.	5.2.3
Including co-products rich in fibre to pig diets may result in a decrease in NH_3 emission but in an increase in CH_4 emitted from manure per pig.	5.2.4
Results are inconsistent about effects of acid supplementation in diets on manure CH_4 emission.	5.3
Some promising results are reported with probiotics and with a combination of tannic acid and sodium fluoride (TANaf), but for the latter one, only *in vitro* results are available.	5.3
Feeding studies using extracts of *Y. Shidigera* or 3-NOP have not investigated the CH_4 emission from pig manure.	5.3
Studies evaluating effects of exogenous enzymes supplemented to the diet do not show effects on CH_4 emission from pig manure.	5.3
The combination of selected technical measures on animal and manure management may reduce the CH_4 and N_2O emissions of pigs by 70%, but these scenario and model studies have to be experimentally verified *in vivo*.	5.4

To explore the potential of nature to improve the quality of life

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