



that reduced CH₄ emission without lowering production. To this end, a database initially developed was updated, compiling data from 61 publications (233 experiments) for various observations in dairy cattle on effects of hydrogen sink (H-sink), ionophore, lipid and concentrate feeds inclusion on enteric CH₄ production, milk production and milk composition from dairy cattle. There was no significant effect (P>0.05) of H-sink and ionophore feeds inclusion on CH₄ production while supplementation of lipid and concentrate considerably suppressed CH₄ production (P<0.05). CH₄ production per kg milk produced was not depressed with H-sink treatment (P<0.05). Lipids lightly increased CH₄ production per kg milk from 26.19 g kg⁻¹ for control to 29.12 g kg⁻¹ for treatment (P>0.05), while concentrate and ionophore feeds inclusion decreased CH₄ production per kg milk with no significant effect (P>0.05). There was a significant effect of concentrate on milk protein and milk yield, which increased from 23.27 kg d⁻¹ for control to 26.52 kg d⁻¹ for concentrate treated diet (P<0.05). Milk yield and milk protein was not significantly affected with Hsink, ionophore and lipid feeds inclusion (P>0.05). This meta-analysis demonstrates that lipid and concentrate feeds inclusion reduced CH₄ emissions from dairy cattle without lowering their production.

KEY WORDS dairy cattle, meta-analysis, methane, methane abatement strategies.

INTRODUCTION

Ruminant farming plays a great role in ensuring global food security and it is able to sustain the livelihood of millions of people in both developed and less developed worlds (Thornton *et al.* 2006). It is economically important with the world's population of about 12% mainly depending on it for their livelihood (Herrero *et al.* 2013; Steinfeld *et al.* 2006). Ruminants utilize poor quality forages as energy source for various life purposes as maintenance, growth and production (Kingston-Smith *et al.* 2010). Unfortunately, ruminants meat and milk production are associated with greenhouse gas (GHG) emissions when compared with the

production of other food types (Williams *et al.* 2009) with great effect on the environment. CH_4 , carbon dioxide (CO_2) and nitrous oxide (N_2O) are the major GHG that promote the effects of solar radiation on the earth surface (Lassey, 2008). It was estimated that up to 18% of anthropogenic GHG emissions globally are mainly from animal agriculture (Steinfeld *et al.* 2006) with enteric CH_4 from ruminant farming systems representing by far the most numerically important source being responsible for circa 60-65% of the CH_4 emissions (USEPA, 2012). Evidently, if the ruminant livestock production is to remain a significant sector in agriculture, effective strategies that minimize CH_4 emission must be devised and implemented which increase

their production efficiency, while at the same time reduce the environmental impact. An important area of focus is therefore to tackle the GHG emissions from livestock production, and to reduce the enteric CH_4 emission from dairy ruminants.

Methane mitigation approaches have been found to be economically and environmentally profitable and advantageous (Gerber *et al.* 2013; Shafer *et al.* 2011). Reduction of CH₄ substantially decreased the amount of GHG associated with milk production (Van Zijderveld *et al.* 2011) with dietary strategies (Martin *et al.* 2010). The policy makers need clear and objective information to get a worldwide picture for recommendations (Veneman *et al.* 2016) which make the qualification of mitigation strategy using meta-analysis of CH₄ emission strategy in dairy sector an important step to identifying the mitigation opportunity and review of new published studies to update data (FAO, 2010). To this end, this meta-analysis study was aimed to identifying the CH₄ mitigation strategy that reduced CH₄ emission without lowering production.

MATERIALS AND METHODS

Data collection/source

The MITIGATE database (Veneman et al. 2016) was initially built and developed in excel compiling data of various treatment details used in enteric CH₄ mitigation strategies from relevant experiments. Data from experiments in dairy cattle were downloaded from this existing database, papers were located and further data on production outcomes were extracted and filled in the database base. The production outcomes data that were investigated and added to the database included quantitative factors as animal body weight, feed details as nutrient composition and chemical composition; milk yield, milk fat, milk protein; rumen fermentation factors, ammonia, nitrogen in urine and faeces. Not all variables were available across all observations in the database. When any of the variables in the study were not reported and it was impossible to calculate the missing data from the reported data, these variables were considered as missing data of the study and were reported as not available (na) in the database.

Updating database

Several literature searches were made using Web of Science, Google Scholar and Aber-Primo (University of Aberystwyth Library Database) to further identify relevant papers on mitigation of CH_4 from dairy cattle from October, 2013 untill August, 2015. Certain keywords as "mitigate", "lipids", "ionophores", "monensin", "tannins", "fatty acids", "saponins" and methane were used. The searches resulted in six publications with 22 experiments and were all *in vivo* studies.

Meta-analysis

The meta-data were subjected to random effects model analysis due to reduced variance of experimental procedure or circumstances which may have been brought about by the vast nature and diversity in animal studies (Hooijmans *et al.* 2014; Vesterinen *et al.* 2014).

The response of CH_4 production, milk production and milk protein to H-sink, ionophore, concentrate and lipid supplementation in dairy cattle for all studies included in the database were evaluated using SPSS package application. The data were analyzed using the following linear model:

$$Y_{ij} = B_0 + B_1 X_{ij} + B_2 X_{ij}^2 + S_i + b_i X_{ij} + e_{ij}$$

Where:

 Y_{ij} : expected outcome i.e. the dependent variable Y observed in the j level of the variable X in the ith study.

 B_0 : overall intercept across all studies equivalent to μ in the random effects model above.

 B_1 and B_2 : overall linear models and quadratic coefficient of Y on X across all studies.

X_{ij}: average value j of the X variable in the ith study.

S_i: random error effect of the ith study.

 b_i : random error effect of the ith study on the coefficient Y on X in the ith study, e_{ij} , S_i and b_i = independent random variables.

eij: residual error.

Results were reported at least square means and standard error of the mean for control and H-sink, ionophore, concentrates and lipid as treatment strategies. It was considered significantly different when $P \le 0.05$.

RESULTS AND DISCUSSION

Description of the MitiGate database

At the time of this study, the MitiGate database (www.mitigate.ibers.aber.ac.uk) was made up of 233 experiments with 61 publications comprising data and significant results of research studies on enteric CH₄ mitigation strategies from all regions of the world in dairy cattle. Ninety-three percent of the research studies were from the year 2000 untill August, 2015 with the largest number of publications from Europe (29 publications), North America (14 publications) and Australasia (14 publications). Nothing was reported for Africa, one publication for South America and three publications were reported for Asia. There was great variation among data on different enteric CH₄ mitigation strategies from dairy cattle from different locations and continents of the world. Table 1 reports the average values of the sample size, animal weight, dietary compositions, dry

matter intake (DMI), CH₄ emissions, milk yield and composition, rumen volatile fatty acids, pH, ammonia concentration, nitrogen excreted in urine and faeces of dairy cattle for the construction of the database used for meta-analysis. The mean concentrations of crude protein (CP) and neutral detergent fiber (NDF) were 184.1 and 408 g kg⁻¹ DM. There was wide range of dietary and animal characteristics evaluated in this study. The DMI varied from 5.5 to 25.7 kg d⁻¹. Methane emissions expressed in g d⁻¹, g kg⁻¹ DMI, GEI MJ MJ⁻¹ also varied greatly in the database.

Table 2 reports the effects of H-sink, ionophores, concentrates and lipids on CH_4 production, milk production and composition. There was no significant difference between the control and the H-sink treated diets (p=0.641) on CH_4 production expressed as (g kg⁻¹ DMI) from dairy cows. Hsink diets did not reduce CH_4 production from dairy cows but significantly increased CH_4 production relative to milk (P<0.05). There was no effect of H-sink supplementation on milk yield and milk protein. Milk yield of 24.35 kg d⁻¹ was reported for cows fed control diet and 25.44 kg d⁻¹ for those fed H-sink supplemented diet while milk protein of 3.07 *vs.* 2.47 was reported for cows fed control diet and those fed H-sink supplemented diet respectively.

CH₄ production was slightly lower (20.59 *vs.* 19.89±1.26 g kg⁻¹ DMI, respectively) for cows fed ionophore supplemented diets than those fed control diets but there was no significant difference (P>0.05). CH₄ production per unit product decreased from 29.63 for cows fed control diets to 25.68 for ionophore supplemented dairy cow diets with no significant effect at (SEM=7.51; p=0.600). There was no effect of ionophore supplementation on milk yield and milk protein (P>0.05). It was reported that 25.23 *vs.* 24.57 ± 2.15 g d⁻¹; 3.02 *vs.* 2.52 ± 1.05% were for cows fed control diet and those fed ionophore supplemented diet for milk yield and milk protein respectively (Table 2).

CH₄ production was significantly lower for cows fed concentrate diets than for those fed control diets (18.26 *vs*. 22.22 \pm 0.83 g kg⁻¹ DMI, respectively (P<0.05). CH₄ production per unit product was lower (23.09 *vs*. 32.22 \pm 4.99 g kg⁻¹, respectively) for cows fed concentrate diets than for those fed control diets but there was no significant difference (p=0.067). There was a significant effect of concentrate inclusion on milk yield which increased from 23.27 for control to 26.52 for treatment \pm 1.23 kg d⁻¹ (P<0.05) and no effect on milk protein (p=0.337) which decreased from 3.02 for cows fed control diets to 2.53 those fed concentrate diet (Table 2).

CH₄ production was significantly lower for cows fed control diets than for those fed lipid-supplemented diets (18.64 *vs.* 21.84 \pm 1.23 g kg⁻¹ DMI, respectively, p=0.009). CH₄ production per unit product increased from 26.19 for

control diets to 29.12 for lipid-supplemented diets with no significant difference at (SEM=7.61; p=0.700) (Table 2).

There was no effect of lipid supplementation on milk yield and protein between dairy cows fed control diets and lipid-supplemented diets (P>0.05).

Meta-analysis of CH_4 mitigation strategies in this study shows the possibility to reduce CH_4 emissions from dairy cattle while not lowering their production if the energy lost as CH_4 by the ruminant can be used for growth and production purposes (Martin *et al.* 2010). Certain regions as Africa, South America and Asia are underrepresented which reflect the state of research and the intensity of livestock systems in these regions.

Differences in the experimental procedure, specific production system where research was carried out and high level of missing data (St-Pierre, 2007) explain the wide variations and diversity of data. These factors made it unbalanced thus prevent the identification of the relationship that exists between treatments and factors of interest. Data was summarized to obtain a qualitative estimation and arrive at a general conclusion for recommendation (Sauvant *et al.* 2008; Hooijmans *et al.* 2014).

Hydrogen sinks (organic acids as nitrates and sulphates) are feed additives used to improve the quality and palatability of ruminants feeds (Shingfield et al. 2002). They provide energy, act as an alternate sink for H₂ in the rumen and inhibit methanogenesis by causing a drop in pH which affects the fermentation of feed (Ungerfeld et al. 2007). Nitrates are reduced to ammonia during metabolism in the rumen and make up for shortage of nutrients in the diet (Ungerfeld and Kohn, 2006; Dijkstra et al. 2007). Nitrates (NO_3) and sulfates (SO_4) accept electrons (alternate hydrogen-sink) and thus reduce methanogenesis. Addition of NO_3^- and SO_4^{-2} reduced CH₄ production in sheep, lactating dairy cows and bulls at a very high dose which may be due to the lower electron available to methanogens. The results obtained agrees with those obtained by van Zijderveld et al. (2010) who reported that nitrate and sulfate supplementation reduced enteric CH₄ production due their ability to favorably utilize H₂ during metabolism of nitrate to ammonia than methanogens. Also, agrees with Veneman et al. (2016) who reported decrease in CH₄ production with Hsink supplementation. Though, H-sink slightly increased CH₄ production but was not significant.

Ionophores as monensin are antimicrobials used as feed additive in ruminant production to improve feed utilization efficiency and animal performance (Moss *et al.* 2000). Experiments with monensin on mitigation of CH₄ in different animal production systems have been studied and reviewed (Grainger *et al.* 2010; Sauer *et al.* 1998; Beauchemin *et al.* 2008).

 Table 1
 Average values of the sample size, animal weight, dietary compositions, dry matter intake, CH_4 emissions, milk yield and composition, rumen volatile fatty acids, pH, ammonia concentration, nitrogen excreted in urine and faeces of dairy cattle for the construction of the database used for meta analysis

Item	Ν	Mean	SD	Min.	Max.
Sample size	214	9.8	8.6	2	53
Animal weight, kg	170	560.3	89.4	295	701
Diet composition, g/kg ¹					
OM	71	912	30.8	786	954
ADF	105	262.9	75.6	103	420
NDF	112	408	142.9	108	1073
СР	148	184.1	57.5	66	399
Fat	58	66.4	80.2	3.8	374
DMI, kg d^{-1}	195	16.9	4.3	5.5	25.7
Methane emission ¹					
g d ⁻¹	213	365.9	125.2	36	671
g kg ⁻¹ DMI	196	20.4	4.8	1.5	32.8
g kg ⁻¹ milk	151	18.9	21.1	1	198.6
GEI MJ MJ ⁻¹	91	0.88	2.2	0.05	7.6
Milk					
Yield, kg d ⁻¹	154	25.2	6.7	8.3	47.4
Milk fat, %	125	4	0.5	1.9	5.5
Milk protein, %	122	3.9	3.9	2.61	29.2
Volatile fatty acids ¹					
TVFAs, mM	51	108.8	19.4	61.5	159.6
Acetate, %	67	50.9	22.8	1.9	96.6
Propionate, %	67	16.9	10	0.1	32.2
Butyrate, %	64	9.8	5.4	0	22.1
РН	57	5.7	1.1	3.5	7.2
NH3, m <i>M</i>	26	10.8	4.4	5.8	19.4
Nitrogen					
Faecal, g d ⁻¹	37	127.7	59.2	0.21	211
Urinary, g d ⁻¹	39	167.4	76.3	0.23	323

SD: standard deviation; N: number; Min: minimum; Max: maximum; OM: organic matter; ADF: acid detergent fibre; NDF: neutral detergent fibre; CP: crude protein; DMI: dry matter intake; GEI: gross energy intake; TVFAs: total volatile fatty acids and NH₃: ammonia.

The results obtained here for ionophore supplementation is in line with the results obtained by Beauchemin *et al.* (2008) who reported that ionophore supplementation of lactating dairy cows diets did not mitigate CH_4 emission. There was no effect of ionophore supplementation on milk yield and protein. This is in disagreement with results obtained by Grainger *et al.* (2010) and Duffield *et al.* (2008) who reported improved milk production with ionophore supplementation.

The long term persistency and inhibitory effects of ionophores on CH_4 production did not consistently reduce CH_4 production (Odongo *et al.* 2007) which may be due to the development of resistance by certain varieties of rumen methanogens and adaptations to antimicrobial treatment (Boadi *et al.* 2004). The public health authority concern over the use of monensin in animal production limits its use which makes it an unviable option for CH_4 mitigation (Martin *et al.* 2010).

It is well known and established (Firkins *et al.* 2001) that feeding concentrates or more energy-dense diets as sugars and starches that are digested in the small intestine provide more energy necessary for production purposes (milk). This, also, reduce enteric CH_4 emission due to the change in rumen pH (<5.5) which reduce the methanogenic populations with increased intake levels (Hegarty, 1999) and decline the ratio of acetate to propionate. The results obtained for concentrate inclusion is in line with those reported by Beauchemin *et al.* (2008).

Though concentrates reduced CH_4 emissions and increased milk yield, it reduced milk quality (protein). High level of concentrate inclusion in diet is needed to achieve positive results but have economic constraints especially in the less developed worlds. High quality forage improvement with high starch levels as cereal forages through breeding and conservation can be used to increase the nutrient use efficiency, productivity of the animal and overall farm profitability thus reduce CH_4 emissions per unit animal product (Beauchemin *et al.* 2008).

Lipids (fats and oils) are supplemented to ruminant diet to increase the energy density of diet for increased production (Shingfield *et al.* 2010). Lipid supplementation reduces CH_4 emissions by reducing the numbers and activities of methanogens and protozoa during metabolism in the rumen (Brask *et al.* 2013).

Item ¹	Ν	Control	Treatment	SEM	P-value
H-sink					
CH_4 (g kg DMI^{-1})	177	19.92	20.56	1.39	0.641
CH ₄ milk (g kg ⁻¹)	143	15.10	40.21	8.79	0.004
Milk yield (kg d^{-1})	144	24.35	25.44	2.45	0.655
Milk protein (%)	112	3.07	2.47	1.25	0.63
Ionophores					
CH_4 (g kg DMI^{-1})	177	20.59	19.89	1.26	0.575
CH ₄ milk (g kg ⁻¹)	143	29.63	25.68	7.51	0.60
Milk yield (kg d^{-1})	144	25.23	24.57	2.15	0.76
Milk protein (%)	112	3.02	2.52	1.05	0.635
Concentrates					
CH_4 (g kg DMI^{-1})	177	22.22	18.26	0.83	0.00
CH ₄ milk (g kg ⁻¹)	143	32.22	23.09	4.99	0.067
Milk yield (kg d^{-1})	144	23.27	26.52	1.23	0.011
Milk protein (%)	112	3.02	2.53	0.51	0.337
Lipids					
CH_4 (g kg DMI^{-1})	177	18.64	21.84	1.23	0.009
CH ₄ milk (g kg ⁻¹)	143	26.19	29.12	7.61	0.70
Milk yield (kg d ⁻¹)	144	26.55	23.24	2.00	0.097
Milk protein (%)	112	2.99	2.56	1.00	0.699

N: number of data and DMI: dry matter intake.

SEM: standard error of the means.

Furthermore, lipid supplementation can influence the palatability, intake of feed, animal productivity and product quality (Odongo *et al.* 2007) which has great implications on the farm. The depressing effect of lipid-supplementation to dairy cow diet on enteric CH_4 production has been reported with variations in the extent of inhibition (Patra, 2013; Beauchemin *et al.* 2008).

Here, lipid feeds inclusion slightly increased CH_4 production but was not significant. The effect of lipid supplementation on CH_4 production agree with the result obtained by Eugene *et al.* (2008) who reported that lipidsupplementation of lactating dairy cow diet could mitigate CH_4 emission by dairy cows.

Certain factors as dose or concentration of lipidsupplementation, duration of feeding, source of lipids (seed *vs.* oil), fat type and diet composition influence the depressing effect of lipid supplementation of dairy cow diet on CH₄ production and productivity (milk production/protein) (Patra, 2013). Long-term experiment to investigate the effectiveness of lipid supplementation as a mitigation strategy is needed.

The physiological stage of animal (lactating *vs.* dry) (NRC, 2001), morphological differences of rumen anatomy and differences in basal diet (feed factors as dose of supplementation, type and source) (Newbold *et al.* 1996) might have influenced the results. The period of the physiological stage (early *vs.* late lactation) of the animal can also influence the response of CH_4 abatement strategy to CH_4 production. During late lactation, decreased milk yield is associated with increased CH_4 yield per unit of product (milk).

This is seen with lipid supplementation while during negative energy balance, when cows are producing at a high level in early lactation, CH_4 production per unit product is reduced (Johnson *et al.* 1996; Chilliard *et al.* 2009) which may explain the decrease in CH_4 milk with concentrate feeds supplementation.

The composition of feed as starch or cell wall carbohydrates, increasing level of feed intake and increasing passage of feed can influence the mitigation strategy and hence affect CH_4 production by strategy per unit of fermentable feed intake (Boadi *et al.* 2004).

In addition, the duration of study influences the effectiveness of the mitigation strategies to reduce CH_4 emission. Short duration of study reduces the effectiveness and persistency of the mitigation strategy under consideration which makes longer-term studies important for better estimation of the efficacy and persistency of abatement strategy (Hristov *et al.* 2013).

The research studies included in the database do not provide a true representation of the real practical farm livestock systems as some studies were carried out under controlled environment, housed, grazed or experimental; some used small number or large number of animals quite different from typical livestock production systems. Some data for incorporation into the database for meta-analysis were missing. Some animals were fed *ad libitum*, restricted and given different feeds as test ingredients which do not represent the true picture of what can be obtained from the different geographical locations of the world (Eckard *et al.* 2010).

Meta-analysis is limited as data from various research studies for analysis are based on or weighted from outcome of previous and similar studies and error that occur due to inability to identify and differentiate between the effects of factors of interest especially when the studies involved are wide (St-Pierre, 2007). Due to differences in study characteristics, environmental and climatic conditions; experimental procedures, species, breed, physiological stage, diet composition, dose of supplementation and specific conditions where research was carried out. In addition, such studies may have considered few factor of importance, which may be small and diverse (Sauvant et al. 2008; St-Pierre, 2007). Thus, the current meta-analysis might not be used to make conclusive reports on the efficacy of CH₄ abatement strategy on animal productivity and product quality or used to make general recommendations as it might not reflect the farming system under consideration. Carrying out research for comparisons between animal species (dairy vs. beef), type (cow vs. heifer), weight, age, duration of study, physiological stage (lactation vs. dry or growth), estimation techniques and the production systems for different geographical regions of the world will increase the effectiveness of mitigation strategies for CH₄ abatement from dairy cows when implemented in specific regions (Sauvant et al. 2008). Finally, measurement and quantification of CH_4 emissions from different herd under different farm conditions will help in the establishment and improvement on the overall estimates and also, properly identify the mitigation strategy that reduce enteric CH₄ productions from dairy cattle under location of interest without lowering their production.

CONCLUSION

There are currently several potential effective strategies to mitigate CH_4 emissions from dairy cattle. The result of this meta-analysis showed that H-sinks, lipid and concentrates are potential and effective strategies resulting in reduced CH_4 emissions from dairy cattle without lowering their production. Many studies used in this meta-analysis only measured CH_4 emissions over a short time frame, which makes longer-term studies important.

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