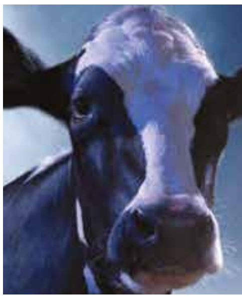

MEGALAC®

Technical Bulletin

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Methane production by ruminants: Effect of rumen-protected fats

Methane is a potent greenhouse gas and there is considerable interest in reducing enteric methane production by ruminants, primarily from an environmental perspective. Rumen-protected fats contribute to reduced methane production, as an additional benefit to supplementation for productivity purposes, primarily through replacement of fermentable organic matter in the diet.

1. Methane and the environment

Methane (CH_4) is one of the three main greenhouse gases (GHG) along with carbon dioxide (CO_2) and nitrous oxide (N_2O). It is highly-potent, having a global warming potential 28-times that of CO_2 (from Zhao and Zhao, 2022). Total global GHG emissions from livestock are estimated to account for 14.5% of total anthropogenic emissions and 40% of this (6% of total anthropogenic GHG emissions) is accounted for by enteric methane from ruminants (from Beauchemin *et al.,* 2020). Most (approx. 90%) of the enteric methane has its origin in the rumen through the process of microbial methanogenesis (Martin *et al.,* 2010). However, methane has a much shorter life-time than CO_2 in the atmosphere (half-life; 8.6 years; Muller and Muller, 2017), which makes it an attractive amelioration target for short-term gains in global warming abatement (Beauchemin *et al.,* 2020).

2. Methane production by ruminants

Methane production is mainly driven by dry matter (DM) intake and fermentability of feed (Beauchemin *et al.,* 2020), incorporating factors such as forage quality and proportion of concentrates in the diet. Increasing DM intake provides greater volume of fermentable feed substrates for production of methane (O'Neill *et al.,* 2012) such that factors influencing DM intake can have positive or negative effects on methane production depending on whether intake is stimulated or reduced.

2.1 Production of methane in the rumen

Methane is produced as a by-product of fermentation of feed components by rumen microbiota (bacteria, protozoa and fungi) to volatile fatty acids (VFA) in the rumen. The fermentation process involves oxidation of reduced co-factors NADH, NADPH and FADH through dehydrogenation reactions resulting in release of hydrogen (H), as well as CO_2 (Martin *et al.,* 2010). This H is then used by methanogenic archaea to reduce CO_2 via the hydrogenotrophic pathway (Beauchemin *et al.,* 2020) leading to formation of methane, as follows :

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

Hence, rumen methanogens have a symbiotic relationship with other rumen microorganisms, using H transferred from the fermentative bacteria, protozoa and fungi to produce methane (from Dai *et al.*, 2022).

Hydrogenotrophic methanogenesis (using H_2 / CO_2 as substrates) is the most widespread of the three major pathways of methanogenesis known (Berghuis *et al.,* 2019), the others being methylotrophic (using methylated compounds) and acetoclastic (using acetate) pathways. Methyl-coenzyme M reductase is the only enyzme present in all types of methanogenesis.

Methane gas produced is eructated by the animal, facilitating removal of excess H from the rumen. It is important to remember that methanogenesis is a natural and essential process within ruminant animals to avoid an accumulation of fermentation-derived H which would otherwise lead to inhibition of dehydrogenase activity involved in the oxidation of reduced cofactors (Martin *et al.,* 2020). Hence, removal of excess H from the rumen through methanogenesis allows NADH to be re-formed to NAD^+, a process essential to the continuation of anaerobic rumen fermentation and microbial growth (from van Zijderveld *et al.,* 2011).

2.2 Effect of fermentative energy source in the rumen

Fermentation of different energy sources in the rumen leads to alternative patterns of VFA production with differential effects on enteric H production. Fermentation of fibrous material in the rumen drives acetate and butyrate which lead to a net release of H, whereas increasing proportion of concentrates in the ration and development of starch-fermenting microbes leads to increased propionate production and reduced H release (Martin *et al.,* 2010).

Production of propionate provides an alternative H sink to methane, reducing the availability of H for methane formation due to competitive requirements for H in the propionate synthesis pathway (Martin *et al.,* 2010). Additionally, increasing concentrate supplementation and starch intake can decrease ruminal pH which also inhibits growth of methanogens (Beauchemin *et al.,* 2020), leading to reduced methane production in the rumen.

3. Energy lost from ruminants as methane

In addition to the environmental implications, ruminal methane production also represents a considerable loss of energy to the animal, ranging from 2 to 12% of gross energy (GE) intake of ruminants (Johnson and Johnson, 1995), though values between 3 and 7% are more realistic in intensive dairy production (Martin *et al.,* 2008). Hence, reducing production of enteric methane without reducing animal productivity is beneficial from both environmental and feed efficiency perspectives.

4. Dietary fat effects on methane production

Fat is an energy-dense nutrient source with many biological functions in animals and is unique among the energy sources available in that it is not fermented to VFA in the rumen as is the case with other nutrients. This characteristic offers a specific method of increasing energy supply without adding to fermentation-induced hydrogen production in the rumen.

4.1 Rumen-active fats

Increasing lipid concentration in the diet has effectively reduced methane production in a number of studies. Beauchemin *et al.* (2020) summarised data from meta-analyses and reported a reduction in methane production (g/d) of between 1 and 5% per 1% increase in dietary fat concentration, with greatest effects achieved from medium chain (C12:0, C14:0) fatty acids and polyunsaturated fatty acids.

Lipids can act to reduce methane production in the rumen by a number of both direct and indirect mechanisms (van Zijderveld *et al.,* 2011; Patra, 2013; Beauchemin *et al.,* 2019), including :

1. Adding fat to a diet replaces a proportion of fermentable organic matter (OM), directly reducing a source of hydrogen formation.
2. Decreasing the metabolic activity and numbers of ruminal methanogens and protozoa.
3. Polyunsaturated fatty acids act as an alternative hydrogen sink via the process of biohydrogenation - though the effect is small, accounting for only 1-2% of total hydrogen use.
4. Reduction of fibre digestibility resulting from an 'oil slick' effect in the rumen.
5. Reduction of fibre digestibility due to toxicity of polyunsaturated fatty acids to strains of fibre-digesting ruminal bacteria. Fibrolytic bacteria are among the most sensitive to inhibition by dietary fats.
6. Via negative effects on DM intake, leading to reduced availability of fermentable substrate.

While beneficial to reducing methane, the negative effects of rumen-active oils on animal production must be considered, in particular the risks of reduced fibre digestibility, feed efficiency and milk fat depression. It's widely recognised that even at low levels of fat supplementation, fats may depress ruminal fibre digestion, or more likely promote formation of fatty acid isomers that cause milk fat depression (Palmquist and Jenkins, 2017).

The negative effects of rumen-active fat sources on productivity limit what can be achieved in terms of methane mitigation without detriment to production and economic aspects at farm level.

4.2 Rumen-protected fats

Rumen-protected fats were developed to overcome the negative effects on rumen fermentation and fibre digestibility associated with unsaturated 'rumen-active' fats (Palmquist and Jenkins, 2017). Their development enabled supplementation of ruminant diets with fat to increase energy density without the negative intra-ruminal and production effects associated with 'free oils', with additional benefits including the ability to deliver a greater proportion of biologically-active unsaturated fatty acids (e.g. C18:1, omega-3) through the rumen to the small intestine.

Rumen-protected fat supplements offer a largely unique approach to methane mitigation efforts. Unlike many of the feed additives evaluated as specific methane inhibitors, dietary fat supplements are established feed ingredients for improving production and fertility aspects in dairy and other ruminant diets. A beneficial effect on methane reduction is achieved more as a 'side-effect' in addition to the performance gains for which these supplements are typically used to enable the producer to gain an economic return.

While rumen-active fats have multiple potential mechanisms by which they can reduce methane production (see Section 4.1), rumen-protected fats, by design, have minimal negative influence on the ruminal environment or microbiota. As such, the primary mechanism for rumen-protected fat-mediated reduction of methane is via direct replacement of fermentable OM in the diet to reduce substrate availability for hydrogen production.

4.2.1 Research with rumen-protected fat supplements

Many studies have evaluated the effects of lipid supplementation of ruminant diets on methane production. However, data on the effects of rumen-protected fats are more limited. The effects of rumen-protected fats should be considered from both production and methane perspectives as beneficial improvements in productivity provide the economic justification for fat supplements, with methane effects an additional benefit.

A number of studies have included methane analyses in their design, incorporating Megalac and other types of rumen-protected fats.

Megalac

i) Andrew *et al.* (1991) provided an early evaluation of the effects of Megalac rumen-protected fat on methane production of lactating and non-lactating Holstein dairy cows. Megalac was added at 2.95% of the diet DM by substitution of ground maize and calcium (targeted to supply approx. 454 g of Megalac/lactating cow/day), resulting in a significant 2.3 kg/cow/d increase in milk yield and a 7.5% reduction in daily methane production (Table 1). These data translate to a 13.7% reduction in methane intensity assessed per kg of milk produced.

Non-lactating cows had similar OM and GE intakes with a tendency for lower methane per unit of GE intake (-5.1%), though the effect did not reach statistical significance.

Table 1: Effect of replacing maize grain with Megalac on methane production (adapted from Andrew *et al.*, 1991)

Production and methane data	Diet		Significance
	Control	Megalac	
Lactating cows			
OM intake (kg/d)	19.1	18.1	$P < 0.05$
GE intake (MJ/d)	393	387	NS
Milk yield (kg/d)	32.0	34.3	$P < 0.01$
CH_4 (litres/d)	540	500	$P < 0.05$ (7.5% reduction)
CH_4 (litres/kg milk)	16.9	14.6	13.7% reduction
CH_4 (% GE intake)	5.43	5.11	$P < 0.05$
Non-lactating cows			
OM intake (kg/d)	4.9	4.9	NS
GE intake (MJ/d)	101	103	NS
CH_4 (% GE intake)	8.2	7.8	NS ($P < 0.10$)

ii) Further data evaluating the effects of Megalac on ruminant methane production were reported by Rapetti *et al.* (2002). In this study, lactating goats (n=6) were offered *ad libitum* silage-based diets with different proportions of alternative energy sources in a Latin square design. Dietary forage : concentrate ratio was close to 1:1 on a DM basis and treatment differences aimed to achieve similar GE concentrations by substituting proportions of maize meal, as follows :

- Treatment (1) : diet containing 32% of diet DM as maize meal (Control)
- Treatment (2) : 23.5% maize meal and 4.7% Megalac
- Treatment (3) : 22.3% maize meal and 9.8% whey permeate.

Gross energy intake and milk yield were similar across the dietary treatments, however goats offered the Megalac-supplemented diet (113 g Megalac/goat/d) produced significantly more milk fat and fat-corrected milk (FCM) than goats offered the other energy sources (Table 2). Energy lost as methane was not significantly reduced by inclusion of Megalac, although a trend toward a reduction in methanogenesis with inclusion of Megalac was observed – accounting for a 9.3% and 17.1% numerically-lower methane loss as a proportion of GE intake compared to the maize and whey permeate diets, respectively.

Table 2: Production parameters of goats offered alternative energy sources (adapted from Rapetti *et al.*, 2002)

Production and methane data	Diet			SEM	Significance
	Maize	Megalac	Whey permeate		
DM intake (kg/d)	2.45^b	2.40^b	2.59^a	0.029	At least $P < 0.05$
GE intake (MJ/d)	44.6	45.4	46.7	0.53	NS
Milk yield (kg/d)	3.37	3.27	3.49	0.062	NS
4% FCM (kg/d)	2.93^b	3.37^a	3.06^b	0.061	At least $P < 0.05$
Milk fat (%)	3.11^b	4.13^a	3.14^b	0.050	$P < 0.001$
Milk fat (kg/d)	0.105^b	0.140^a	0.111^b	0.0025	$P < 0.001$
Milk protein (%)	2.93	3.03	2.98	0.030	NS
Milk protein (kg/d)	0.097	0.099	0.102	0.0015	NS
CH_4 energy (% GE intake)	7.5	6.8	8.2	0.18	NS

Values in the same row with different superscripts are significantly different

iii) Beck *et al.* (2019) evaluated the effect of supplementing diets of grazing beef cattle (n=20; mean initial live weight 269 kg) with rumen-protected (Megalac) or rumen-active (whole cottonseed and soyabean oil) fat sources on production and methane parameters. Methane was measured using an automated head-chamber space system (GreenFeed) for the 59-d of the study.

Cattle were offered concentrate supplements at the rate of 1.59 kg/d, with the Megalac and soya oil ingredients included in the concentrate to supply approx. 330 g/d of each fat source to provide the same amount of supplemental lipid as the whole cottonseed treatment. Dietary fat concentration increased from 2.4% of DM in the Control (non-supplemented) diet to between 5.5 and 6.4% of DM in the treatment groups.

Inclusion of fat supplements increased energy intake and liveweight gain, though only the rumen-active fat sources reduced ($P < 0.01$) daily methane production (g/d) (Table 3). However, inclusion of Megalac in the diet resulted in significant reductions in methane production per kg liveweight gain by 54.5%, per MJ of GE intake by 17.5% and per MJ of digestible energy (DE) intake by 21.6%.

Table 3: Effect of different fat supplements on performance and methane production of grazing beef cattle (Beck *et al.*, 2019)

Production and methane data	Treatment diet				SEM	Significance
	Control	Whole cottonseed	Soyabean oil	Megalac		Control vs fat supplements
Intake and production parameters						
DM intake (kg/d)	6.5	7.0	7.3	7.0	0.30	0.04
GE intake (MJ/d)	117.2	136.1	138.6	135.7	5.00	< 0.01
DE intake (MJ/d)	55.7	68.7	72.9	68.2	3.30	< 0.01
Liveweight gain (kg/d)	0.45	0.65	0.92	0.93	0.080	< 0.01
Methane emissions						Control vs Megalac
g/d	200	175	177	202	9.0	NS
g/kg liveweight gain	466	316	168	212	44.0	< 0.01
g/kg DM intake	31.2	24.9	24.5	28.9	1.90	NS
% GE intake	9.7	7.1	7.3	8.0	0.60	0.03
% DE intake	20.4	14.4	14.0	16.0	1.40	0.01

Other rumen-protected fats

i) van Zijderveld *et al.* (2011) evaluated the effects of a saturated rumen-protected 'high-C16' fatty acid supplement (approx. 330 g/d intake) compared to a fat source containing a blend of C8:0 and C10:0 fatty acids, or a polyunsaturated source containing extruded linseed (mainly C18:3). Supplements were offered isolipidically to the Control ('high-C16') diet and animals were restrictively-fed to avoid effects of potential differences in DM intake due to fat supplementation. Diallyl disulphide, a component of garlic oil, was included as an additional treatment.

Milk yield and measures of methane were similar between treatments, indicating that the rumen-protected 'high-C16' supplement had similar methane mitigating effects to the other fat sources, including the rumen-active highly-polyunsaturated extruded linseed supplement (Table 4). The requirement for supplements to be evaluated *in vivo* was also noted, given that the trial ingredients had previously shown beneficial effects *in vitro.*

Table 4: Effect of replacing a 'high-C16' fat source with other fat and additive sources (van Zijderveld *et al.*, 2011)

Production and methane data	Diet supplement				SEM	Significance
	'High-C16' control	Extruded linseed	C8/C10 blend	Diallyl disulphide		
Production parameters						
DM intake (kg/d)	16.5	16.9	16.7	16.8	0.21	NS
Milk yield (kg/d)	24.4	25.4	22.3	24.8	1.01	NS
Milk fat (%)	4.82[a]	4.47[a]	5.38[b]	4.52[a]	0.155	< 0.001
Milk protein (%)	3.41	3.33	3.59	3.40	0.078	NS
Methane emissions						
g/cow/d	371	394	388	386	26.1	NS
g/kg DM intake	23.2	23.2	23.2	22.9	1.22	NS
g/kg milk	15.8	16.0	18.2	15.5	1.83	NS
% GE intake	6.3	6.4	6.6	6.4	0.15	NS

Extruded linseed and C8:0 / C10:0 fatty acid blend replaced the 'high-C16' supplement in the Control diet isolipidically

ii) Alstrup *et al.* (2015) evaluated the effects of supplementing lactating Danish Holstein dairy cows (n=12) with either cracked rapeseed, or with rumen-protected fat variants included at 2.3% of diet DM (approx. 500 g/d intake for the standard rumen-protected fat), at intervals through lactation from 48 to 212 days in milk. Dietary fat concentration was increased from 2.6% of DM in Control to 5.6% of DM in the treatment diets.

As presented in Table 5, DM and net energy of lactation (NEL) intakes were similar, though cows offered the fat supplements produced significantly more milk and produced less methane per unit of DM intake, and per unit of GE intake (by 9.0%). Addition of fat increased production of energy-corrected milk (ECM) compared to Control, but the effect did not reach statistical significance, probably due to the low numbers of animals used. These authors also noted that the reduction in methane production when fat was added to the ration persisted throughout lactation, an important finding in relation to effectiveness on methane suppression over time.

Table 5 : Effects of different fat supplements on production and methane parameters of lactating dairy cows (Alstrup *et al.*, 2015)

Production and methane data	Diet supplement				Significance
	Control	Whole cracked rapeseed	Rumen-protected fat #	Rumen-protected fat with HMBi ##	
Production parameters					
DM intake (kg/d)	22.4	19.3	21.6	22.2	NS
NEL intake (MJ/d)	152	135	150	157	NS
Milk yield (kg/d)	30.3	34.9	37.2	43.0	0.01
Milk protein (%)	3.89	3.46	3.44	3.37	< 0.001
Milk fat (%)	4.75	4.58	4.64	4.27	NS
ECM (kg/d)	29.9	32.9	35.3	38.5	NS
Methane emissions					
Litres/d	669	588	622	564	NS
Litres/kg DM intake	30.6	29.8	28.5	25.6	0.04
Litres/kg ECM	24.2	17.7	17.4	14.9	NS
% GE intake	6.53	6.20	5.94	5.35	0.03

Rumen-protected fat = blend of 40% calcium salt of palm fatty acids and 60% hydrogenated palm fatty acids (fatty acid profile 43% C16, 25% C18:0,16% C18:1, 3.6% C18:2 - % DM)   ## MetaSmart = hydroxy-methionine-analog-isobutyrate

iii) Morris and Kononoff (2021) evaluated production responses of lactating Jersey cows in a multi-treatment design study with varying levels of fat, starch and supplemental lysine. Fat was increased by inclusion of a rumen-protected fat ranging from 0 up to 4% of diet DM in replacement for soya hulls. The fatty acid profile of the supplement offered was 57.2% C16:0, 20.8% C18:0, 12.8% C18:1 (% of total fatty acids).

Selected data for three different fat concentration treatments, offered at common starch and lysine supplementation, are presented in Table 6. Rumen-protected fat intakes were 0, and approx. 396 and 776 g/d, at 0, 2 and 4% inclusion rates, respectively.

Energy lost as methane and total daily methane production decreased linearly with increasing dietary fat concentration, recording a 16.6% reduction from the lowest to highest fatty acid concentrations in the study. Lower methane production also contributed to increased efficiency of conversion of DE to ME as dietary fat concentration increased.

Table 6: Effect of increased rumen-protected fat supplementation on performance and methane production of lactating Jersey cows (adapted from Morris and Kononoff, 2021) #

Production and methane data	Rumen protected fat addition to diet (% DM)			SEM
	0	2	4	
Total diet fatty acids (% DM)	3.0	4.6	6.2	
Production parameters				
DM intake (kg/d)	20.5	19.8	19.4	0.68
GE intake (Mcal/d)	83.4	83.5	83.7	2.86
DE intake (Mcal/d)	54.6	55.2	53.2	2.44
ME intake (Mcal/d)	47.9	49.1	47.4	2.27
ME / DE	0.876	0.889	0.891	0.005
Milk yield (kg/d)	27.9	28.9	29.0	1.41
Milk fat (%)	4.95	5.14	5.19	0.352
Milk fat yield (kg/d)	1.372	1.474	1.502	0.0856
Milk protein (%)	3.61	3.44	3.39	0.107
Milk protein yield (kg/d)	1.005	0.991	0.980	0.0419
Methane emissions				
Mcal/d	4.17	3.72	3.48	0.207
Litres/d	440	391	367	2.86

Diet treatments selected at common starch concentration (25.7% of DM) and lysine inclusion (8.9 g/d)

4.2.2 Persistency of effects

Effect of methane inhibitors can be influenced by the risk of adaptation of ruminal microflora over time, such that efficacy of such inhibitors may be reduced (from Alstrup *et al*., 2015). van Zijderveld *et al*. (2011) noted that while many dietary strategies have been proposed to decrease methane production in ruminants, few have shown a persistent decrease of methane *in vivo*.

However, this is unlikely to occur where methane is reduced by addition of rumen-protected fat to a ration given that the primary mechanism involves a reduction in fermentable OM in the rumen. As such, the methane mitigation effect resulting from increasing fat addition to rations is hypothesised to be persistent (Alstrup *et al.,* 2015).

4.2.3 Effects on fibre digestibility

As noted, the development of rumen-protected fats aimed to avoid the negative effects on fibre digestibility caused by supplementation of diets with liquid oils and high-oil by-products.

However, in addition to minimising disruption of rumen fibre digestibility, recent data indicate that some rumen-protected fats may actually improve NDF digestibility, potentially mediated by stimulatory effects of palmitic acid on strains of fibre-digesting bacteria. Meta-analyses data by dos Santos Neto *et al*. (2021a,b) reported a 4.5% (absolute) increase in NDF digestibility when lactating cow diets were supplemented with 'high-C16' (>80% C16:0) fatty acid supplements (mean 1.81% of diet DM), and a lesser but significant response with calcium salts of palm fatty acid distillate (PFAD) (1.6% absolute increase) (mean inclusion 2.20% of diet DM).

These data indicate that strains of fibrolytic rumen microbiota may have ability to use fatty acids from rumen-protected fat supplements. This feature would favour growth and population increase, promote fibre digestibility, and benefit efficiency of nutrient use (dos Santos Neto *et al*., 2021b). However, the corollary is that fermentation of additional fibre components could increase production of methane and negate a portion of the benefit from the replacement of fermentable OM with a rumen-protected fat supplement.

4.2.4 Fertility effects

Rumen-protected fats can improve fertility by a number of mechanisms, including increasing energy supply, increasing progesterone production and improving quality and survivability of fertilised eggs.

Davies *et al.* (1992) reported a reduction of 9.4 days open when Megalac was incorporated into diets of lactating cows (range approx. 255 to 330 g/d), accompanied by a 1.6 kg/d increase in milk yield. Similarly, Garcia-Bojalil *et al.* (1998) reported an increase in pregnancy rate from 52 to 86% when lactating dairy cows were offered Megalac at 450 g/cow/d for 120-days post partum, as well as a mean 1.6 kg/d milk yield response.

Improved fertility traits can translate to fewer replacement animals needed which has major implications on methane production at farm level. Garnsworthy (2011) evaluated the effect of replacement rate and milk yield on total methane emissions from dairy herds (Figure 1) and determined that the proportion of total methane emissions produced by replacements can be reduced from 30% at poor fertility levels (oestrous detection 50%; conception rate 30%) to 10% under improved fertility conditions (oestrous detection 70%; conception rate 60%).

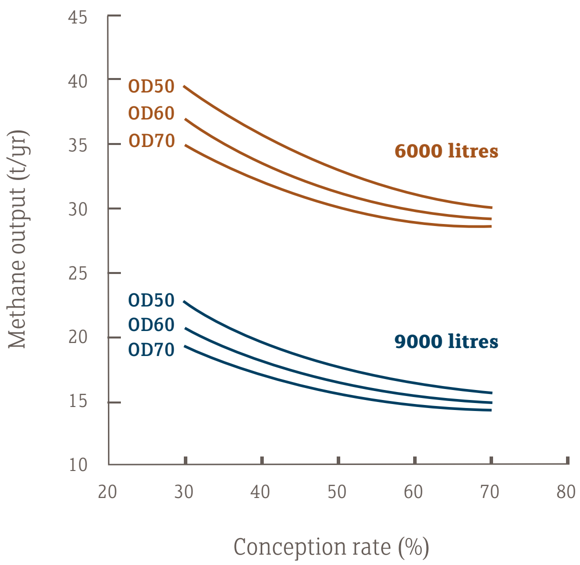

Figure 1: Annual methane output in dairy herds with varying conception rate and oestrous detection (OD) rate of 50,60 or 70% producing 1 million litres of milk/annum and average milk yield of 6,000 or 9,000 litres/cow (Garnsworthy, 2011)

The considerably lower methane output from the high yielding (9,000 litre) herd (Figure 1) results from a combination of fewer cows to produce a similar volume of milk and lower forage : concentrate ratio in the diet of these higher-producing animals. Improved fertility, resulting in less time spent in late lactation and dry period when higher forage, methane-stimulating diets are typically offered, also contributes to lower methane emissions from herds (Garnsworthy, 2011).

These data indicate the importance of considering both production and fertility effects when evaluating the potential beneficial effects on methane production through supplementation of diets with rumen-protected fats.

Summary

- Methane is a potent greenhouse gas, having 28-times the global warming potential of CO_2.
- Ruminants produce methane as a result of ruminal fermentation of feed and is typically higher with high-fibre rations.
- Rumen-protected fats provide energy and are not fermented in the rumen, reducing the volume of fermentable substrate for methane synthesis.
- Research data demonstrate that improvements in animal performance and fertility, with concomittant reductions in methane production, can be achieved when rumen-protected fats are incorporated into diets.

The Methane Molecule