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Literature survey of the influence of dietary fat composition on methane production in dairy cattle

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Abstract

The quantitative influence of dietary fat composition on methane production in dairy cows was estimated from a literature survey involving thirty-seven diets from seven papers. With this database covering a large range for animal performances and dietary lipid composition, it seems that methane production (expressed as l/kg DMI of feed) decreased when either the level of production of the animals or the dietary ether extract content increased. Moreover, the database analysis pointed out that the methane decrease was only due to the fatty acid part of the ether extract and allowed to statistically quantifying this diminution which was proportional to the degree of unsaturation of the fatty acids. This compilation of experiments allowed the proposal of an index of unsaturation to improve the estimation of methane production in vivo by ruminants. The addition of unsaturated fats might be of interest for decreasing methane production, but it also might have adverse effects, such as the increase of feed refusals or the reduction in cellulose digestion.

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1. Introduction

Several authors have pointed out that dietary fat alters the ruminal microbial ecosystem and, in particular, the competition for metabolic hydrogen between the methane and propionate production pathways (Demeyer et al., 1969; Czerkawski, 1972; Fonty and Morvan, 1996). Among domesticated

animals, cattle bred in intensive conditions are one of the biggest methane producers when considering the total methane produced by different species (Crutzen et al., 1986; Johnson and Johnson, 1995; Mathison et al., 1998; Sauvant et al., 1999). Moreover, fatty feeds seem to be the most promising dietary alternative to synthetic methane inhibitors (Jouany, 1994; McAllister et al., 1996; Spears, 1996).

Although ruminant methanogenesis has been related to dietary composition in numerous works (Sauvant et al., 1999; Demeyer and Fievez, 2000), it seemed interesting to quantitatively evaluate from a

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literature survey the influence of dietary fat composition on methane production in dairy cows. Thus, it must be kept in mind that most of the methane produced by ruminants has a ruminal origin and that the part coming from the hindgut digestion is known to be less methanogenic (Demeyer and Fievez, 2000).

The unit generally used by energeticians for methane is the percentage of gross energy lost as CH₄. It seemed however more convenient for our purposes, and afterwards for formulation, to express methane production as l/kg DM feed, in order to allow for direct comparison of diets from a methanogenic point of view.

2. Materials and methods

Seven papers were retained for this study (Coppock et al., 1964; Jentsch et al., 1972; Schiemann et al., 1972; Krishna Mohan et al., 1975; Van der Honing et al., 1981, 1983; Holter et al., 1992). For the 37 diets considered, the composition in ingredients was precisely known.

For each ingredient, the composition of its ether extract (EE) was estimated by using the CVB (1996) tables. We used these tables to separate the ether fraction into two: a fatty acid one and a non-fatty acid one. In the fatty acids fraction, we considered the following acids (C_{12:0}, C_{14:0}, C_{16:0}, C_{16:1}, C_{18:0}, C_{18:1}, C_{18:2}, C_{18:3} and $\Sigma C_{\geq 20}$), which were usually indicated in the tables. When feedstuffs were missing in this database, we used the French AFZ (2000) database. The composition of Megalac[®] (Holter et al., 1992) was obtained from the Internet site of Church and Dwight which produced this component. For forages, we used the literature compilation of Morand-Fehr and Tran (2001).

The $\Sigma C_{\geq 20}$ term corresponds to a sum of the

different fatty acids that have between 20 and 26 atoms of C in their chain. A few of them is saturated and the rest is unsaturated (PUFAs). In this sum, the different acids might have then very different nutritional meanings.

The SAS package (SAS, 1987) was used for statistical calculations. Regressions were performed to study the relationships between parameters. The link between the dependent variable and the one or more independent variables was analysed using the residual standard deviation (R.S.D.) of the dependent variable. A principal component analysis (PCA) was used to examine the relationships among several quantitative variables, as described by Lebart and Fenelon (1971).

3. Results

The database covered a large range for dry matter intake, as well as for milk and methane production (Table 1). The diets also had quite different ether extract (EE) contents which did not have the same composition of fatty acids (Table 2). The oleic acid (C_{18:1}) was the most important of the fatty acids (32% w/w of total fatty acids) ranging between 7 and 51% of the total quantity of fatty acids. Linoleic acid (C_{18:2}) represented around 28% of total fatty acids and palmitic acid (C_{16:0}), 17%. The fourth major fatty acid was linolenic acid (C_{18:3}) representing 13%.

The mean production of methane, expressed as litres per kg dry matter intake (DMI), was negatively related by a curvilinear function to the DMI expressed as grams per kg live-weight (Fig. 1):

$$\text{CH}_4 \text{ l/kg DMI} = 44.9 - 0.0220 \text{ DMI}^2$$

$$(r^2 = 0.58, n = 37, \text{R.S.D.} = 3.42 \text{ l/kg DMI}) \quad (1)$$

Table 1
Main production parameters

	Mean	S.D.	Minimum	Maximum
Dry matter intake (g/kg live-weight)	26.1	3.4	18.4	32.0
Live-weight (kg)	554	47	390	625
Milk production (l/day)	20.1	6.0	8.5	35.2
Methane production (l/kg DM)	29.6	5.2	15.9	37.9

Table 2
Main statistical parameters of the diets

	DM (%)			
	Mean	S.D.	Minimum	Maximum
Ether extract	4.41	3.27	1.24	11.40
Total fatty acids	3.49	3.04	0.70	10.05
C _{12:0}	0.026	0.077	0.000	0.320
C _{14:0}	0.043	0.076	0.001	0.271
C _{16:0}	0.595	0.681	0.108	2.686
C _{16:1}	0.042	0.072	0.001	0.263
C _{18:0}	0.233	0.441	0.014	1.711
C _{18:1}	1.124	1.533	0.027	5.769
C _{18:2}	0.954	0.888	0.118	3.006
C _{18:3}	0.458	0.265	0.172	1.225
ΣC _{≥20}	0.014	0.019	0.000	0.076

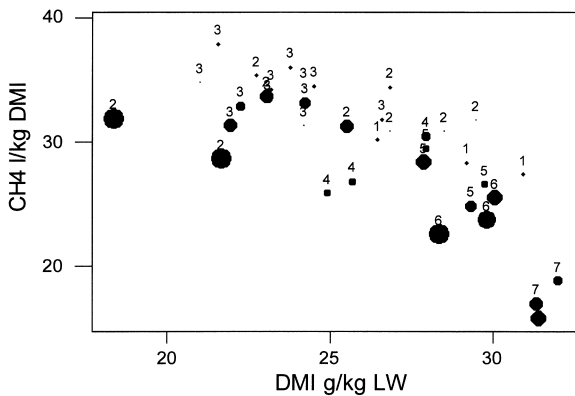


Fig. 1. Relationship between methane production and dry matter intake. Size of symbols is proportional to ether extract content of diets. Numbers referred to the paper: ¹Coppock et al., 1964, ²Jentsch et al., 1972, ³Schiemann et al., 1972, ⁴Krishna Mohan et al., 1975, ⁵Van der Honing et al., 1981, ⁶Van der Honing et al., 1983, ⁷Holter et al., 1992.

This relationship can be improved by taking into account the dietary EE content (expressed as percentage DM)

$$CH_4 \text{ l/kg DMI} = 47.3 - 0.0212 \text{ DMI}^2 - 0.680\% \text{ EE} \quad (r^2 = 0.76, n = 37, \text{R.S.D.} = 2.62 \text{ l/kg DMI}) \quad (2)$$

In Fig. 1, the sizes of the symbols were proportional to the dietary EE content. Eq. (2) demonstrates that an increase of 1% of dietary EE decreased the methane production of 0.68 l/kg DM of feed. The other chemical parameters (crude fibre, crude protein, ash or nitrogen free extract) did not bring any

improvement in the reduction of the residual standard deviation of these two Eqs. (1) and (2).

We partitioned the dietary EE content into its fatty acid and non-fatty acid (NFA) parts. The latter consists of an unsaponified portion (sterols, pigments . . .) and of the saponified part made of NFAs (glycerol, galactose, phosphoric acid, amines, . . .) (Morand-Fehr, 1981). The percentage of NFAs in the EE content was 28.5 (±13.0) and decreased curvilinearly as the EE increased (Fig. 2):

$$\% \text{NFA/EE} = 49.1 - 6.65\text{EE} + 0.295\text{EE}^2 \quad (r^2 = 0.71, n = 37, \text{R.S.D.} = 7.2\%) \quad (3)$$

This means that in diets with a low EE content, the NFA portion was more important than in diets high in EE, and that for the same EE content, diets rich in forages, especially green forages, had lower fatty acids percentages than diets high in concentrates. The percentage of forage improved this relationship:

$$\% \text{NFA/EE} = 32.0 - 5.86\text{EE} + 0.261\text{EE}^2 + 0.287\% \text{ forage} \quad (r^2 = 0.84, n = 37, \text{R.S.D.} = 5.3\%) \quad (4)$$

In fact, the methane production decrease was only due to the fatty acid part of the EE, as the precision

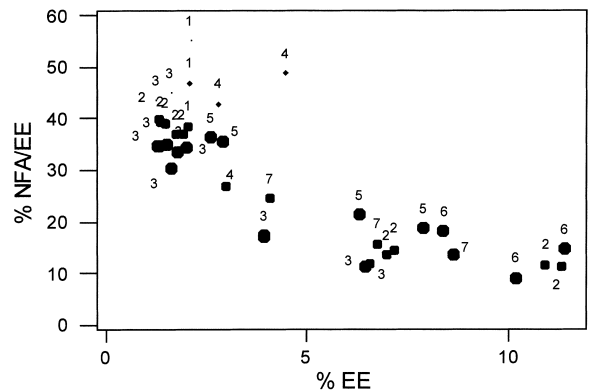


Fig. 2. Relationship between ether extract content and its percentage of non-fatty acids. Size of symbols is proportional to percentage of concentrate in the diets. Numbers referred to the paper: ¹Coppock et al., 1964, ²Jentsch et al., 1972, ³Schiemann et al., 1972, ⁴Krishna Mohan et al., 1975, ⁵Van der Honing et al., 1981, ⁶Van der Honing et al., 1983, ⁷Holter et al., 1992.

of prediction was of the same magnitude with ether extract (Eq. (2)) or with its fatty acid part (Eq. (5)):

$$\begin{aligned} \text{CH}_4 \text{ l/kg DMI} &= 47.2 - 0.0217\text{DMI}^2 \\ &- 0.735\% \text{ FA/kg DM} \\ (r^2 &= 0.76, n = 37, \text{R.S.D.} = 2.61 \text{ l/kg DMI}) \quad (5) \end{aligned}$$

When the total quantity of fatty acids was partitioned into the sum of saturated fatty acids ($\Sigma C_{:0} = C_{12:0} + C_{14:0} + C_{16:0} + C_{18:0}$), of monoene fatty acids ($\Sigma C_{:1} = C_{16:1} + C_{18:1}$), of $C_{18:2}$, of $C_{18:3}$ and of longer fatty acids with a chain with 20 C or more ($\Sigma C_{\geq 20}$), the latter fatty acids had a very big influence, and the $C_{18:2}$ was also statistically significant:

$$\begin{aligned} \text{CH}_4 \text{ l/kg DMI} &= 45.0 - 0.0180\text{DMI}^2 \\ &- 1.84 C_{18:2} - 84.2 \Sigma C_{\geq 20} \\ (r^2 &= 0.81, n = 37, \text{R.S.D.} = 2.37 \text{ l/kg DMI}) \quad (6) \end{aligned}$$

The high correlation between $C_{18:2}$ and $\Sigma C_{:1}$ ($r^2 = 0.46$, $n = 37$, **, $P < 0.01$) and between $C_{18:2}$ and $C_{18:3}$ ($r^2 = 0.45$, $n = 37$, $P > 0.01$) might explain the lack of specific effect of $\Sigma C_{:1}$ and $C_{18:3}$. Moreover, the quantity of $C_{18:2}$ was higher than that of $C_{18:3}$, and its standard deviation was as much as three times higher than that of $C_{18:3}$ (0.888 vs. 0.265). As DMI^2 and $\Sigma C_{\geq 20}$ were significantly correlated ($r^2 = 0.30$, $n = 37$, **, $P < 0.01$), the values for these coefficients had to be considered with caution from a statistical point of view.

Therefore, we calculated an index of unsaturation as the sum of the quantity of each of the unsaturated fatty acids multiplied by its degree of unsaturation. The mean value of this index was 4.45, with a S.D. of 3.43 and values between 1.44 and 13.98. We obtained a precision for the prediction of methane emissions similar to the preceding one:

$$\begin{aligned} \text{CH}_4 \text{ l/kg DMI} &= 46.3 - 0.0188\text{DMI}^2 \\ &- 0.545 \text{ index} - 91.1 \Sigma C_{\geq 20} \\ (r^2 &= 0.84, n = 37, \text{R.S.D.} = 2.16 \text{ l/kg DMI}) \quad (7) \end{aligned}$$

In this last equation, the ether extract content, the total quantity of fatty acids, the quantity of saturated fatty acids or the percentage of forage could not be added with any precision.

Other methods of expression were tested, such as the quantities of the different fatty acids expressed as percentages of the total fatty acid content or of the EE content. The best way of expression remained the index of unsaturation.

In all the residuals of these equations, there was no significant effect due to the source of data.

We performed a principal component analysis (Lebart and Fenelon, 1971) in order to better understand the relationships between variates. We used DMI^2 , percentage of forage, quantities of FA and the index of unsaturation. The first axis explained 44.2% of the total variation. It showed an opposition between methane production and quantities of saturated fatty acids and of $\Sigma C_{\geq 20}$ (Fig. 3). The relative proximity of methane production and percentage of forage is not due to a significant correlation between these variates ($r^2 = 0.38$, $n = 37$, $P = 0.65$), but to the usually low ether extract content of forage, and thus of the quantities of fatty acids, especially of saturated ones. This would appear on the third axis. The second axis explained 23.6% of the total variation. It is a kind of 'unsaturation' axis, which means that saturated acids are opposed to unsaturated ones, and that the opposition is enhanced with the degree of unsaturation. When considering diets, those that were rich in maize (and especially corn gluten feed) and animal tallow had lesser methane production than the others. The diets rich in unsaturated fatty acids contained mainly rapeseed oil or soybean hulls.

The plan with axes 1 and 3 showed the opposition between methane emission, on one hand, and dry matter intake and the quantity of long chain fatty acids ($\Sigma C_{\geq 20}$), on the other hand (Fig. 4). The

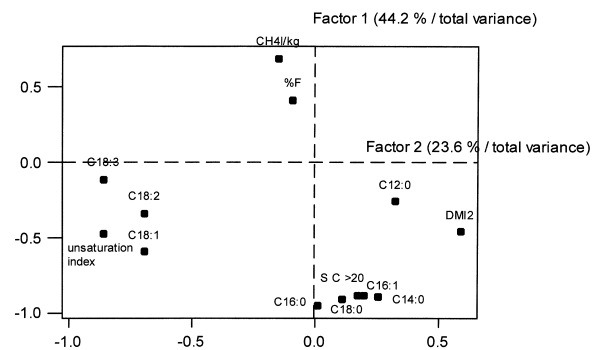


Fig. 3. Principal component analysis (plan 1–2, variates).

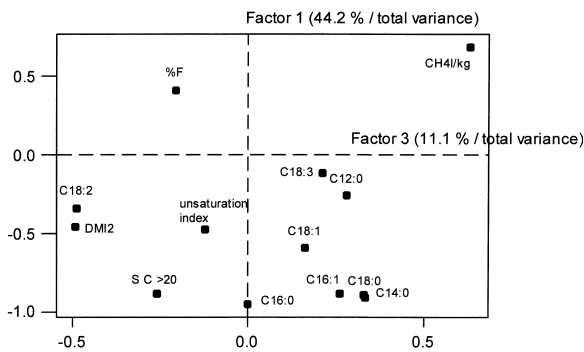


Fig. 4. Principal component analysis (plan 1–3, variates).

opposition between methane emission and DMI indicated that methane emission decreased with DMI, or, for the studies considered, with level of milk production. Moreover, the experiments with the highest milk production (Holter et al., 1992; Van der Honing et al., 1981, 1983) differed mainly for their $C_{18:2}$ intakes which were higher than for the other experiments. The third axis differentiated the percentage of forage and methane emission (see above).

4. Discussion

The main objective of this survey was to compile *in vivo* results from different laboratories in order to find general laws about the influence of dietary fat composition on methane production in dairy cows. The precision for the methane production was quite good, even if numerous strong hypotheses had been made: we used data from published tables to estimate the composition of fatty acids because this information was not given, and probably not measured in the papers. In the CVB tables, the fatty acid composition was similar for cakes and oils which needed to be confirmed. Moreover, the precision of the analysis for the shortest fatty acids ($C_{12:0}$ – $C_{16:0}$) or for the longest ones ($\Sigma C_{\geq 20}$) may have been poor.

Some of the obtained results were in agreement with the literature despite the fact that we used a different method of expression for them (l/kg DM vs. % of gross or digestible energies lost as methane): the methane production decreased when either the level of intake (Hoffmann et al., 1972; Giger-Reverdin et al., 2000) or the ether extract

content increased (Hoffmann et al., 1972; Van Nevel and Demeyer, 1995; Giger-Reverdin et al., 1992).

The ether extract is made up of a fatty acid part and of an unsaponified part. Our results quantify the decrease of methane production as a function of the fatty acid part only. Moreover, the stability of the coefficients for DMI in the different equations indicated the specific effect of this fatty acid part.

It is well known that the presence of long-chain PUFAs inhibits methane production in the rumen through two ways: provision of an alternative metabolic H acceptor to reduction of CO_2 and direct toxic effects on ruminant microorganisms (Czerkawski et al., 1966b; Demeyer et al., 1969; Broudiscou et al., 1994; Johnson and Johnson, 1995; Dong et al., 1997). However, the amount of total metabolic H used in the biohydrogenation process of endogenous saturated fatty acids (1%) is small compared with that used for reduction of CO_2 to methane (48%), VFA synthesis (33%) and bacterial cell synthesis (Czerkawski, 1986). Our results concerning the decreasing effect of $C_{18:2}$, and our index of unsaturation which corresponds to the quantity of hydrogen bonds available for CO_2 reduction, agreed with these hypotheses. These are also confirmed by the lack of effect of the quantity of saturated fatty acids. Our results were in agreement with *in vitro* observations (Nieman, 1954; Demeyer and Henderickx, 1967) who concluded that the inhibition of Gram-positive bacterial growth increased with the number of double bonds they contained, and with the *in vivo* observations of Czerkawski et al. (1966a) on sheep with several unsaturated C_{18} supplementations.

The high correlation between DMI^2 and $\Sigma C_{\geq 20}$ ($r=0.55$, $n=37$), such as the low values of $\Sigma C_{\geq 20}$ did not allow any conclusion to be drawn about the specific effect of this type of fatty acid which was badly defined in the tables. Nevertheless, most of the fatty acids which are contained under this broad term $\Sigma C_{\geq 20}$ should be highly unsaturated with three or more double bonds, like the arachidonic acid ($C_{20:4}$). This could explain the big influence of this sum in the variations of CH_4 emissions (Eqs. (6) and (7)). It is therefore of importance that the composition of fatty acids would be more precisely given in the feed tables, especially for the long chain fatty acids. The lack of effect of medium-chain fatty acids (lauric and myristic acids) might be due to their quite low levels

as their sum was around 2% of the sum of all acids, but *in vitro* they have a depressive effect on methane production when their sum was higher than 20% (Dohme et al., 2000).

From a practical point of view, some side effects of fat consumption must be kept in mind: if the amount of fat is too large, it increases the amount of refusals (7% as fed for coconut oil in sheep, Machmüller and Kreuzer, 1999). Lipids depress fibre degradation in the rumen and total tract (Doreau et al., 1991; Ferlay and Doreau, 1992); this decrease depends upon the nature and the amount of lipid as well as the animal species and experimental conditions. For example, a momentarily high concentration of the FA in rumen fluid might be more important in suppressing methane than its constant presence (Czerkawski et al., 1966b, Sauer et al., 1998). They also modified the fermentescibility of feeds and shifted the volatile fatty acid composition to a propionate basis (Demeyer et al., 1969). Finally, the addition of lipids is costly and it must be measured against the energy available to the animal (Mathison et al., 1998)

5. Conclusion

As domestic ruminants are responsible for about 12.5% of total global methane emissions (Crutzen, 1995), it should be of interest to find a dietary means to decrease methane production. Provided that it does not disturb the microbial ecosystem too much, the supplementation of unsaturated fatty acids should be considered.

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