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A Meta-Analytic Approach to Predict Methane Emissions from Dairy Goats Using Milk Fatty Acid Profile

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Abstract: The aim of this work was to develop an equation to predict methane yield (CH_4, g/kg dry matter intake) from dairy goats using milk fatty acid (FA) profile. Data from 12 research papers (30 treatments and 223 individual observations) were used in a meta-regression. Since most of the selected studies did not extensively report milk fat composition, palmitic acid (C16:0) was selected as a potential predictor. The obtained equation was: CH_4 (g/kg dry matter intake) = 0.525 × C16:0 (% in milk fat). The coefficient of determination (R^2 = 0.46), the root mean square error of prediction (RMSPE = 3.16 g/kg dry matter intake), and the concordance correlation coefficient (CCC = 0.65) indicated that the precision, accuracy and reproducibility of the model were moderate. The relationship between CH_4 yield and C16:0 content in milk fat would be supported by the fact that diet characteristics that increase the amount of available hydrogen in the rumen for archaea to produce CH4, simultaneously favor the conditions for the synthesis of C16:0 in the mammary gland. The obtained equation might be useful, along with previous published equations based on diet characteristics, to evaluate the environmental impact of dairy goat farming.

Keywords: methane; goats; milk; fatty acids

1. Introduction

Goat farming is considered an important source of income and nutrition for poor and marginal farmers around the world. The resilience of this species to harsh conditions is particularly remarkable, but their enteric methane (CH_4) emission along with productive performance should be taken into account in the changing climate scenario [1]. The livestock sector is responsible for 14.5% of the global anthropogenic greenhouse gas emissions (GHG), 44% in the form of CH_4 and the remaining part almost equally shared between nitrous oxide (N_2O) and carbon dioxide (CO_2). Small ruminants (sheep and goats) contribute 6.5% of total livestock sector emissions of GHG, with over 55% in the form of CH_4 from enteric fermentation [2].

Several equations to predict enteric CH_4 emission from dairy cows using animal and diet characteristics as well as milk fatty acid (FA) profiles have been developed through meta-analyses of published research [3–6]. The most common predictors in those equations are body weight, milk yield and composition, dry matter intake (DMI), diet composition, and the contents of iso FA and rumen biohydrogenation-derived FA in milk fat [3–6]. The basis of the proposed equations is the direct relationship between diet and rumen microbiota and the intrinsic connection between the lipid metabolism of ruminal bacteria and the milk FA profile [7]. Some equations for predicting CH_4 emission based on animal and diet characteristics have been also proposed for goats at any

productive stage [8]. However, to our knowledge no predictive equations based specifically on milk FA profile are available for dairy goats. Direct comparisons of milk FA responses to the same diets show dissimilarities between dairy cows and goats [9,10], which supports the assumption that the equations obtained in dairy cows cannot be used as proxy to predict CH_4 emission from dairy goats. A meta-analysis of published research data obtained in dairy goat studies could help to quantitatively relate CH_4 emission to milk FA profile in these animals, as an alternative to the scarcity of knowledge on the matter. Therefore, the objective of this study was to perform a meta-analysis and a meta-regression to quantify the relationship between CH_4 and milk FA profile in dairy goats.

2. Materials and Methods

Research papers on dairy goats were searched in Google Scholar using the operator "allintext:" and introducing the keywords: "methane", "milk fatty acids" and "goats". The search was limited to the year 2000 onwards. Studies were selected if they simultaneously reported milk FA profile (g/100 g FA) and CH_4 yield (g/kg DMI) or CH_4 production (g/d or L/d) along with daily DMI. Any treatment where CH_4 emission was manipulated by administering chemical additives to the animals was excluded. Twelve peer reviewed research papers that involved a total of 30 treatments with 223 individual observations fulfilled the requirements (Table 1) [11–22]. The experimental diets were very varied and included hays, silages, agro-industrial by-products, cereals, protein concentrates and fat sources. In the selected studies, CH_4 emission was measured using the sulfur hexafluoride technique (n = 1), open-circuit respiration chamber (n = 6) and open-circuit mask system (n = 5). All the studies determined the FA contents in milk fat by gas chromatography.

Table 1. Details of the 12 experiments included in the meta-analysis.

Author	n [1]	Method [2]	Breed [3]	DIM [4]	Forage Type
[11]	9	RC	M-G	57	Alfalfa hay Alfalfa + oat hay
[12]	6	RC	M-G	60	Alfalfa hay + olive by-product silage Alfalfa hay + tomato surplus silage
[13]	10	OCM	M-G	LL	Alfalfa hay
[14]	10	OCM	M-G	LL	Alfalfa hay Maralfalfa hay
[15]	10	OCM	M-G	LL	Alfalfa pellets Lemon leaves pellets
[16]	10	OCM	M-G	ML	Alfalfa pellets Orange leaves pellets
[17]	6	SF_6	AxB	n.s.	Berseem hay
[18]	8	OCM	M-G	ML	Alfalfa hay
[19]	8	RC	M-G	13	Alfalfa hay
[20]	3	RC	S	106	Italian ryegrass silage
[21]	3	RC	S	106	Native pasture Pasture hay Non-forage diet
[22]	8	RC	M-G	ML	Alfalfa hay

[1] Number of animals per treatment. [2] Method of CH_4 emission measurement (RC: Respiratory chamber; OCM: Open-circuit mask; SF_6: Sulphur hexafluoride). [3] M-G: Murciano-Granadina; AxB: Alpine x Beetal; S: Saanen. [4] Days in milk (LL: Late lactation; ML: Mid lactation; n.s.: Not specified).

The data on diet composition, experimental design, number of animals per treatment, body weight, DMI, milk yield, milk fat, milk fat FA profile, molar proportions of volatile fatty acids (VFA) in rumen fluid and CH_4 emission reported in the studies were recorded and stored in a Microsoft Excel spreadsheet. The factors to convert units were 0.71 g/L CH_4 and 55.65 kJ/g CH_4 [8]. Since most of the selected studies did not extensively report milk fat composition, the milk fat contents of several FA that are highly related to CH_4 emission were not available [3,6,23]. Thus, we decided to select palmitic

acid (C16:0) as a potential predictor because of its positive relationship with CH_4 emission reported in the literature [3,23] and its negative relationship with dietary factors that are known to alter the rumen environment, like feeding high concentrate diets, elevated consumption of unsaturated FA or both [9,10,24,25].

All the statistical analyses were performed with the MIXED procedure of SAS University Edition 3.8 (SAS Institute, Cary, NC, USA). A regression analysis (meta-regression) was conducted to study the prediction of CH_4 yield (g/kg DMI) using C16:0 content (%) in milk fat [26]. Visual inspection of the data showed that the relationship between the variables was linear (Figure 1), thus a linear regression was performed. The statistical model included the fixed effect of C16:0 content in milk fat and the random effects of study and study × C16:0 content interaction. The data were weighed with the square root of the number of animals used in each treatment using the WEIGHT statement. Statistical significance was declared at $p < 0.05$.

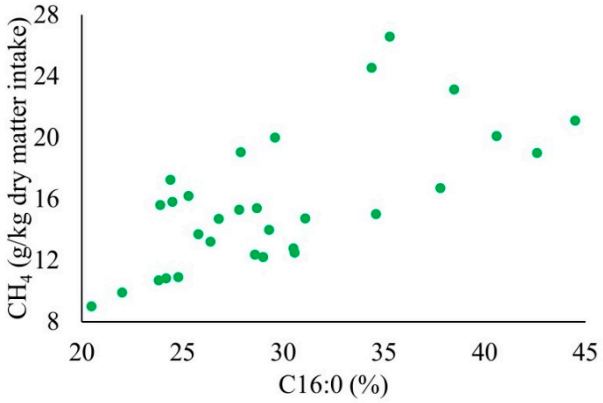

Figure 1. Plot of observed methane (CH_4) yield vs. palmitic acid (C16:0) content in milk fat.

3. Results

The descriptive statistics of the data compiled from the studies included in the meta-analysis are shown in Table 2. Despite the uniformity in the breeds used in the studies that were included in the dataset (Table 1), body weight, dry matter intake, milk yield and composition, and CH_4 emission showed considerable variation. The ranges of intakes of diet components varied widely, which reflected the substantial differences between diets in the studies. The correlation between CH_4 yield and proportion of C16:0 in milk fat showed that both were positively linearly related not only in the pooled data (Figure 1) but also within nine out of the 11 experiments with two or more treatments.

Table 2. Descriptive statistics of the data compiled from the studies included in the meta-analysis.

	Mean	Minimum	Maximum	Standard Deviation
Body weight (kg)	44.9	34.0	55.0	6.07
Dry matter intake (kg/d)	1.75	0.99	2.69	0.443
CP [1] intake (kg/d)	0.30	0.18	0.47	0.076
NDF [2] intake (kg/d)	0.57	0.32	1.15	0.205
EE [3] intake (kg/d)	0.06	0.01	0.19	0.041
NFC [4] intake (kg/d)	0.61	0.20	1.01	0.262
Milk yield (kg/d)	1.92	0.94	3.69	0.831
Milk fat (%)	4.86	2.96	6.90	1.103
Palmitic acid (% in milk fat)	29.8	20.5	44.5	6.20
Methane (g/d)	28.1	12.3	68.8	14.33
Methane (g/kg dry matter intake)	15.7	9.0	26.6	4.36

[1] Crude protein. [2] Neutral detergent fiber. [3] Ether extract. [4] Non fibrous carbohydrates.

The intercept of the obtained regression equation was not significant ($p = 0.82$) and was removed from the model. The slope of the final no-intercept regression Equation (1) was highly significant ($p < 0.001$) and the coefficient of determination (R^2) and the root mean square error of prediction (RMSEP) indicated that the goodness of fit and the accuracy of the model were moderate. Neither the mean bias (1.49 g/kg DMI; $p = 0.62$) nor the linear bias (−0.089 g/kg DMI; $p = 0.64$) were significant, thus almost all the model prediction error was due to random variation (Figure 2). Moreover, the bias correction factor, which reflects accuracy, was 0.96 and the concordance correlation coefficient or reproducibility index was 0.65.

$$\begin{gathered} CH_4 \text{ (g/kg DMI)} = 0.525\ (\pm 0.0252) \times \text{C16:0 (\% in milk fat)} \\ R^2 = 0.46;\ \text{RMSEP} = 3.16 \text{ g/kg DMI } (20.1\%) \end{gathered} \quad (1)$$

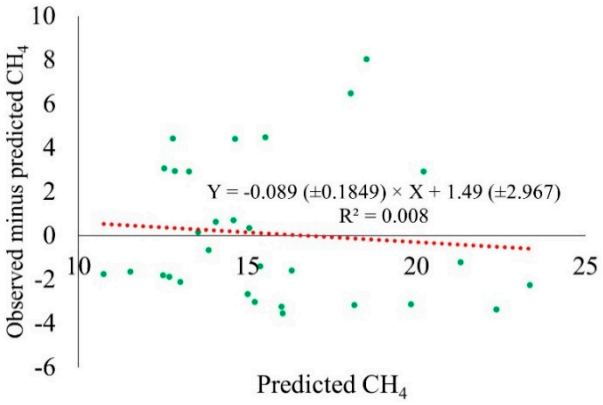

Figure 2. Plot of observed minus predicted vs. predicted methane yield (CH_4, g/kg dry matter intake).

Those results aimed to further evaluate model adequacy. However, the number of studies in the dataset was not large enough to split them into development and evaluation subsets. Therefore, as a substitute for assessing the model performance, we decided to compare CH_4 yield predicted using Equation (1) or using the relationship between CH_4 yield and the molar proportions of VFA (acetate, propionate and butyrate) in rumen fluid as described in recent research [27]. The results from nine studies (21 treatments and 187 individual observations) in the dataset that reported molar proportions of VFA in rumen fluid were used for the comparison [11–16,18,19,22]. The descriptive statistics of the variables used in the calculations and of the predicted CH_4 yields according to both methods are shown in Table 3.

Table 3. Descriptive statistics of the data in the nine studies that were used to compare methane (CH_4) yield predicted using either palmitic acid content in milk fat ($CH_{4C16:0}$) or molar proportions of volatile fatty acids (VFA) in rumen fluid (CH_{4VFA}).

	Mean	Minimum	Maximum	Standard Deviation
Palmitic acid (% in milk fat)	29.4	20.5	44.5	6.77
Acetate (mol/100 mol VFA)	62.1	53.5	67.6	4.23
Propionate (mol/100 mol VFA)	15.4	11.1	26.7	3.73
Butyrate (mol/100 mol VFA)	16.8	10.6	22.4	2.72
CH_4 (g/kg dry matter intake)	14.8	9.0	21.1	3.37
$CH_{4C16:0}$ (g/kg dry matter intake)	15.4	10.8	23.4	3.55
CH_{4VFA} (g/kg dry matter intake)	11.6	6.7	14.0	2.07

As shown in Table 4, all the measures of precision (Pearson r), accuracy (root mean square error of prediction, RMSPE; and bias correction factor, BCF) and reproducibility (concordance correlation coefficient, CCC) of CH_4 yield prediction were better when applying Equation (1) than when using the

molar proportions of VFA as predictors. Moreover, the latter method underpredicted mean CH_4 yield as ~22%, whereas Equation (1) overpredicted of mean CH_4 yield as ~4%. It is noticeable that none of the models showed mean or linear bias, which indicated that almost all the prediction error was due to random variation in both cases.

Table 4. Prediction analysis of methane (CH_4) yield in nine studies using either palmitic acid content in milk fat ($CH_{4C16:0}$) or molar proportions of volatile fatty acids in rumen fluid (CH_{4VFA}) as independent variables.

	RMSEP [1] g/kg DMI	RMSEP %	Mean Bias	Linear Bias	BCF [2]	Pearson r	CCC [3]
$CH_{4C16:0}$	2.36	16	3.49 $p = 0.13$	−0.27 $p = 0.07$	0.98	0.77	0.76
CH_{4VFA}	4.41	30	6.62 $p = 0.11$	−0.29 $p = 0.39$	0.54	0.43	0.23

[1] Root mean square error of prediction. [2] Bias correction factor. [3] Concordance correlation coefficient.

4. Discussion

The number of studies, treatments and individual observations (12, 30 and 223, respectively) was lower and the ranges of body weight, DMI and CH_4 yield in our dataset were narrower than in previous research, where 42 studies that involved 211 treatments and 978 individual observations were used and the ranges of body weight, DMI and CH_4 yield in the dataset were 14.5 to 58.7 kg, 0.34 to 2.35 kg/d and 6.5 to 39.0 g/kg DMI, respectively [8]. Those differences are due to the fact that our study was limited exclusively to lactating dairy goats and only papers that reported simultaneously CH_4 emission and C16:0 content in milk fat were included.

The ruminal contents of VFA have been related to CH_4 emission using stoichiometric equations because the proportions of acetate, propionate and butyrate determine the amount of hydrogen available for utilization by archaea [27,28]. However, our results showed that CH_4 yield predicted using Equation (1) would be more precise, accurate and reproducible than predictions using the molar proportions of VFA in rumen fluid (Table 4), at least under conditions similar to those used in the experiments included in the dataset (Table 1).

The role of C16:0 as a predictor of enteric CH_4 emission would be supported by current scientific knowledge. First, acetate production from carbohydrate fermentation by rumen cellulolytic bacteria is inextricably linked to methanogenesis because it is the main source of hydrogen to be combined with carbon dioxide by archaea to release CH_4 [29,30]. Second, acetate and to a lesser extent β-hydroxybutyrate are the main substrates for de novo synthesis of C16:0 in the mammary gland [31]. Third, approximately 50% of C16:0 incorporated into milk fat comes from de novo synthesis [31,32]. Fourth, rumen cellulolytic bacteria are very sensitive to dietary changes, especially those which imply low ruminal pH, high intake of polyunsaturated FA (PUFA) or both [33–35]. Finally, in connection with the latter, the increased availability of long-chain unsaturated FA (either absorbed intact from the diet or derived from incomplete rumen biohydrogenation of dietary PUFA) to the mammary gland alters the activity ratio of the key enzymes acetyl-CoA carboxylase and fatty acid synthetase involved in de novo synthesis of FA [24]. Therefore, any diet modifications that negatively affect rumen cellulolytic bacteria would result in lowered availability of both hydrogen for archaea to produce CH_4 and acetate for the mammary gland to synthesize de novo C16:0, and, in most cases, would also impair the activities of the enzymes involved in the latter. As a result, CH_4 emission and C16:0 content in milk fat would decrease simultaneously [3,23].

Despite our results, other milk FA are expected to be better predictors of CH_4 emission in dairy ruminants than C16:0 [3,6,23]. Odd and branched-chain FA from microbial origin and *trans* FA arising from incomplete ruminal biohydrogenation of dietary unsaturated FA have aroused much attention in the last decade because they are directly related to rumen bacteria and their responses to diet characteristics [36,37]. Cellulolytic bacteria are rich in iso FA [38] and are the main responsible of

ruminal biohydrogenation of dietary unsaturated FA [39]. A decline in the abundance of cellulolytic bacteria promotes a shift in rumen biohydrogenation pathways with the increased formation of *trans*-10, *trans*-12 C18:2 and other biohydrogenation intermediate FA that have been associated with milk fat depression [40]. Unfavorable ruminal conditions to cellulolytic bacteria not only will diminish CH_4 emission due to the shortage of hydrogen for archaea but also will decrease the contents of iso FA and rise the contents of *trans* FA in milk fat due to a lower bacterial growth and a more incomplete rumen biohydrogenation process. These effects are well reflected in the predictive equations developed for predicting CH_4 emission from dairy cows (e.g., iso C16:0 adds up and *trans*-10 C18:1 and *trans*-11 C18:1 subtract in Van Lingen's equation [6]). Principal component analyses of FA contents in caprine milk fat would suggest that, at least, iso C14:0, iso C16:0 and *trans*-11 C18:1 could be potential predictors of CH_4 emission from dairy goats [41,42].

Applying Equation (1) to published results obtained in dairy goats, it can be predicted that the consumption of 30 g/d of linseed oil would decrease CH_4 yield by ~14% and the reduction would rise up to ~40% at an intake of 125 g/d [41,42]. Those predicted changes are in line with the findings in dairy cows that were fed increasing levels of linseed fat [43]. Furthermore, from the original data of published research [44], Equation (1) predicted that CH_4 yield would diminish ~18% after the first 72 h from the inclusion of 48 g/d of linseed oil in the diet of dairy goats.

5. Conclusions

As more research papers that simultaneously report enteric CH_4 emission and extensive milk FA profile in dairy goats become available to develop better predictive equations through meta-regression, a simple equation based on C16:0 content in milk fat might be useful, along with previous published equations based on diet characteristics, to evaluate the environmental impact of dairy goat farming.

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