Correlation and Estimation of Methane Yield with Volatile Fatty Acid Profiles in Holstein Ruminal Fluid from In Vivo Studies

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Abstract. Methane yield (MY) in dairy cows was influenced by the cow breed used. However, the prediction of MY from previous studies has yet to use a specific breed of cow. This study analyzed the relationship between methane emissions per unit of dry matter intake (methane yield) based on volatile fatty acids (VFA) profile in the rumen of the Holstein breed from several in vivo studies and their estimation and validation. The database was created using different literature from search engines and included 53 studies from 18 articles. The articles were selected based on published in vivo studies of the Holstein breed with metrics such as MY and VFA profile such as acetate (A), propionate (P), Butyrate (B), and ratio acetate to propionate (A:P). The data obtained were analyzed using Pearson correlation and multiple linear regression to estimate the MY. The VFA profile concentration with significant correlation ($P<0.05$) was used to estimate MY yield using multiple linear regression. The model obtained will be validated using the mean absolute deviation (MAD), root mean square error (RMSE), and mean absolute percentage error (MAPE). The findings revealed a significant ($P<0.05$) and very significant ($P<0.01$) correlation between the VFA profile and MY. MY was estimated using the following equation: MY= 117.896 + 0.091A - 2.438P - 1.703B - 9.964(A/P). It was concluded that the methane yield (MY) can be estimated from the VFA profile in the rumen of the Holstein breed.

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1 Introduction

The release of methane (CH_4) from the digestive process of ruminant animals is a significant source of greenhouse gas (GHG) emissions, which pose a severe risk to global warming [1]. Methane is a highly potent greenhouse gas, possessing a global warming potential far more significant than carbon dioxide [2]. The production of enteric methane during the anaerobic fermentation of dietary carbohydrates in the rumen results in the host losing energy and also contributes significantly to greenhouse gas emissions. The production of methane can lead to a decrease in energy, which in turn can have a negative impact on the efficiency of energy usage in ruminant animals. It can affect their growth rate, feed efficiency, and nutrient utilization [3].

Holstein cattle, a predominant dairy breed, have been extensively studied for their methane emissions, providing a rich dataset for analysis. Research has delved into various aspects related to methane emissions in Holstein cattle, including genetic analysis [4], rumen microbiota composition [5], rumen methanogen populations [6], and genome-wide association studies [7]. Methanogenesis, the process of producing methane in the rumen, is influenced by various factors, one of which is volatile fatty acid (VFA) profiles [8]. Studies have shown that the presence of protozoa in the rumen can impact methanogenesis, with the elimination of protozoa leading to changes in ruminal VFA profiles and methane production [8]. The rumen methanogenic community plays a crucial role in methane production, with the hydrogenotrophic pathway being the most common mechanism in mature rumens [9].

The volatile fatty acids profile (VFAs) serves as primary energy sources for ruminants, accounting for up to 70% of their energy requirements [8]. The synthesis of VFAs, a primary product of microbial fermentation that provides a crucial energy source for the animal, is primarily driven by diet characteristics [10]. Acetate is the predominant VFA, followed by propionate and butyrate. The ratio of these VFAs can significantly impact methane production. Volatile fatty acids (VFA) and trace elements (TEs) influence methane production and VFA metabolism in positive and negative ways during methanation [11]. The ruminal hydrolysate provided a substrate rich in volatile fatty acids (VFA) for direct use in the methanogenic process [12].

This study aimed to analyze and estimate methane yield based on VFA profiles, specifically in Holstein cattle, using data from in vivo studies. This study utilized a comprehensive database focusing on in vivo methane yield measurements and VFA profiles in Holstein cattle. The selection criteria included only studies with detailed VFA and methane yield data. Pearson correlation analysis determined the relationship between individual VFAs (acetate, propionate, butyrate) and methane yield. Multiple linear regression was used to develop an estimation model for methane yield based on significant VFA correlations.

2 Material and Method

A total of 53 studies from 18 articles [13–28]. were utilized as a database. The data was collected by searching for articles published in Scopus on the in vivo technique of measuring methane production from specific dairy cow breeds (Frisian Holstein) without any interventions or treatments. The database comprised the volatile fatty acids profile, including acetate (A), propionate (P), butyrate (B), the ratio of acetate to propionate (A/P), and methane yield (MY). The obtained data undergoes a data cleansing step prior to analysis. Table 1 displays the descriptive statistics for the methane yield and the profile of volatile fatty acids utilized in the database.

Table 1. Descriptive statistics of database.

Parameter	N	Mean	Minimum	Maximum
Methane yield (g/kg DMI)	53	21.74	9.69	36.40
Acetate (%)	53	62.01	52.70	68.80
Propionate (%)	53	21.63	12.40	34.80
Butyrate (%)	53	12.16	5.90	19.74
Acetate:Propionate	53	3.04	1.55	5.48

Note: DMI, dry matter intake.

The gathered data undergoes a data cleansing procedure to guarantee uniformity and dependability. The Pearson correlation coefficient was employed to determine the relationship between the VFA profile and the parameters to be evaluated, specifically the MY. The factors in the volatile fatty acids profile that showed a significant connection ($P<0.05$) were utilized to estimate MY by applying the multiple linear regression approach. The variables that did not show a significant correlation were removed from the estimation model since their high probability values indicate that they do not contribute to explaining the variation of MY. The application of Multiple Linear Regression [29] using the following mathematical model (equation 1):

$$Y = \alpha + \beta 1x1 + \beta 2x2 + \cdots + \beta nxn + \mathcal{E} \quad (1)$$

where Y is MY, α is a constant, β is estimation coefficient regression from an independent variable, x is an independent variable, namely the volatile fatty acids profile, n is volatile fatty acids profile content (1, 2, …), and Ɛ is an error.

The validation of the MY estimation was performed by comparing the values of the mean absolute deviation (MAD) [30], root mean square error (RMSE) [29], and mean absolute percent error (MAPE) [31] between the MY estimation model and the observed values. The validation measures are used to assess the correctness and robustness of the MY estimate model. The data is analyzed using Review Manager 5.4 and IBM SPSS version 25.

3 Result and Discussion

The correlation matrix presented in **Figure 1** illustrates the relationships between various volatile fatty acids (VFA) profiles (acetate, propionate, butyrate, and the acetate to propionate ratio) and methane yield (MY) in Holstein dairy cows. Pearson's correlation coefficients were calculated to determine the strength and direction of these relationships. Correlation analysis was conducted to explain the presence and extent of the relationship between the VFA profile and methane yield. The analysis revealed a negative correlation between methane yield in Holstein cows with propionate ($P < 0.001$) and butyrate ($P < 0.01$). It suggested that higher concentrations of propionate and butyrate are associated with lower methane yields. Conversely, there was a positive correlation between methane yield with acetate and the acetate-to-propionate ratio ($P < 0.001$). It indicated that higher acetate levels and a higher acetate-to-propionate ratio are associated with increased methane yields.

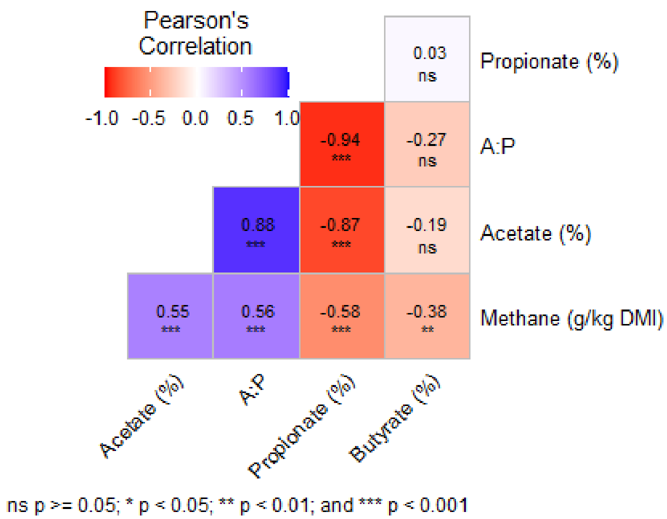

Figure 1. Coefficient of correlation between volatile fatty acids (VFA) profile and methane yield (n = 53).

These findings align with the biochemical pathways of rumen fermentation, where acetate production is typically associated with higher methane emissions. Acetate production involves pathways that generate more hydrogen, which is then utilized by methanogens to produce methane. Methanogens create methane by reducing CO_2 with hydrogen or catalyzing acetate fermentation [32]. Acetate and hydrogen availability affects methanogenic pathways, with syntrophic acetate oxidation producing methane under specific conditions [33]. In addition, methane production in the rumen is positively correlated with the acetate and propionate ratio, indicating methane production [34]. Furthermore, the shift in rumen fermentation from acetate to propionate production has been observed to reduce methane emissions by decreasing hydrogen availability in the rumen [35].

Propionate, on the other hand, acts as a hydrogen sink, thus reducing the availability of hydrogen for methane production. Propionate acts as an alternative hydrogen sink, diverting hydrogen away from methane synthesis [36]. Furthermore, the modulation of rumen microbiota by certain compounds can lead to an increase in lactate-consuming bacteria and a higher molar proportion of propionate, further emphasizing the role of propionate in mitigating methane emissions [37].

Table 2. Prediction equation MY and validate with published equation of MY.

Model	Regression Equation	MAD	RMSE	MAPE	Sig.	R	R^2
Methane Yield (g/Kg DMI)	MY= 117.896 + 0.091 A - 2.438 P -1.703 B-9.964 (A/P)	3.42	4.23	20.13%	0.000	0.748	0.560

Note: DMI, dry matter intake; MY, methane yield; A, acetate; P, propionate; B, butyrate; MAD, mean absolute deviation; RMSE, root mean square error; MAPE, mean absolute percentage error.

Table 2 shows that the regression analysis revealed that the model is statistically significant ($p < 0.001$), with an R^2 value of 0.560. It indicated that 56% of the variability in methane yield can be explained by the VFA profiles included in the model. The correlation coefficient (R) of 0.748 suggests a strong positive relationship between the observed and predicted methane yields. The MAD and RMSE values of 3.42 and 4.23, respectively, indicate that the model's predictions are reasonably accurate, with low deviation from the observed values. The MAPE value of 20.13% suggests that, on average, the predicted methane yield deviates from the observed yield by about 20.13%. Although the model demonstrates promising predictive ability, further refinement is still possible to enhance accuracy and minimize prediction errors. It could involve integrating additional variables or utilizing more sophisticated modelling techniques.

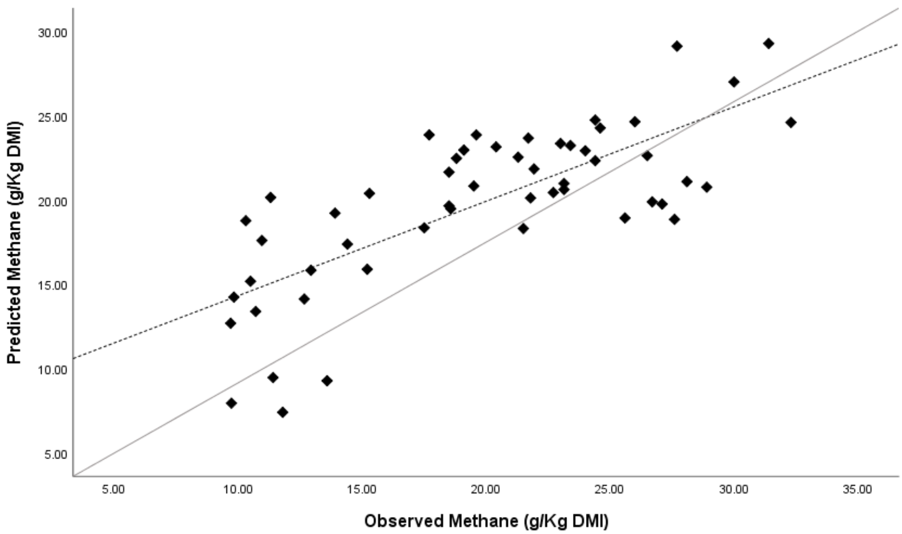

Figure 2. Scatter plot of observed vs. predicted methane yields in holstein dairy cows.

The scatter plot visually represents the correlation between the actual and expected methane production, as seen in Figure 2. The visualization results indicate that the calculated MY values align with the observed MY values. The scatter plot demonstrates a direct relationship between the observed and anticipated methane yields, suggesting that the regression model established in this study can accurately forecast methane emissions using VFA profiles. The dashed line shows the regression line that predicts MY based on observed MY. The proximity of the data points to the regression line indicates a strong alignment with the model, showcasing its capacity to make accurate predictions. The observed disparities from the line of equality suggest differences between the expected and observed values, which may be attributed to unaccounted elements in the model.

4 Conclusion

The methane yield (MY) in Holstein dairy cows can be estimated from the VFA profiles. The estimation equation is MY = 117.896 + 0.091 A - 2.438 P - 1.703 B - 9.964 (A/P), where A represents acetate, P represents propionate, B represents butyrate, and A/P is the acetate-to-propionate ratio. This equation can be utilized for diet formulation in ruminants to predict and manage methane emissions effectively.

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