Comparison of enteric methane emissions using respiration chambers and the Cornell Net Carbohydrate and Protein System model predictions

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Abstract

In collaboration with Cornell University, the Environmental Defense Fund (EDF) conducted an analysis to corroborate the accuracy of methane emissions estimations, using feed chemistry inputs and animal characterization, from the Cornell Net Carbohydrate and Protein System (CNCPS, Van Amburgh et al., 2015). The CNCPS is a nutritional model that estimates nutrient requirements of cattle and therefore, widely used for diet formulation in the dairy industry in the United States and worldwide. Because the model follows the principles of the energy system, it also possesses the capability to estimate methane emissions through gaseous losses. Therefore, to assess the validation of the enteric methane predictions in the model, we conducted an analysis comparing a subset of data collected from direct gas measurements from respiration chambers (RC) against predicted methane output generated by CNCPS. The dataset included a total of 8 Holstein pregnant heifers that undergone RC measurements across 5 consecutive measurement periods. For model predictions, inputs pertaining to heifer characterization (e.g., body weight, age, days pregnant, feed intake), and dietary components (e.g., feed chemistry and neutral detergent fiber [NDF] digestibility) were utilized as inputs for the predictions. Input data was averaged to the group level for animal characterization (n = 8) and averaged weekly for dietary components. Statistical analysis was performed in R statistical software using a paired t-test. Results highlighted no evidence that measured methane production from RC differed from predicted methane emissions generated by CNCPS ($P > 0.05$).

Background

Methane is 81.2 times more potent than carbon dioxide in a 20-year lifespan (Mar, 2022), driving urgent need for methane mitigation. Farm-level measurement, monitoring, reporting, and verification (MMRV) can help quantify and isolate methane emissions derived from enteric fermentation, which make up 27% of the US dairy's greenhouse gas footprint (Pelton, 2025). A landscape of measurement tools is developing to help the dairy supply chain identify hotspots and reduce emissions. Among those tools, are respiration chambers (RC), which are regarded as the "gold standard" for biological measurements. However, these can be quite impractical to be applied on a large scale. In this context, the development and use of other tools that can accurately predict enteric methane emissions becomes extremely important for the industry.

The Cornell Net Carbohydrate and Protein System (CNCPS) is a model that was developed to estimate nutrient requirements, feed utilization, animal performance and excretion of cattle. The model uses detailed knowledge about feed composition, digestion, and metabolism to ensure nutrient requirements are met. The model is used to formulate rations by approximately 60% of dairy nutritionists in the United States (Prestegaard-Wilson, 2021), and is distributed across 60 countries. The CNCPS incorporates equations for both carbon dioxide (CO_2) and methane (CH_4) emissions (Van Amburgh et al., 2015). The equations from Casper and Mertens (2010) for CO_2 and Mills et al. (2003) for CH_4 were tested within the CNCPS and demonstrated good accuracy and precision at predicting enteric carbon dioxide and methane emissions.

Thus, the CNCPS model may also be leveraged as an MMRV tool to aid in baseline quantification of methane inventories. For farmers and nutritionists, this means reports on methane can be automatically generated without creating additional work streams. Given its high adoption rate and preexisting capacity to predict enteric methane emissions from cattle, there is an opportunity for existing MMRV tools to utilize outputs from CNCPS in their systems.

This report aims to highlight the data alignment between enteric methane measurements performed using RC with the estimations generated by CNCPS.

Methods

Data collection

The data utilized for the current analysis is a subset from a study conducted at Cornell University. Briefly, 8 pregnant Holstein dairy heifers (18 ± 0.49 months of age [mean ± SD], 164 ± 4.42 days carrying calf, and 556 ± 39.4 kg of body weight [BW]) were enrolled. Prior to RC measurements, heifers were acclimated to the research facility and were trained to use the RC system. At the end of acclimation and training periods, heifers were divided equally into 2 groups and underwent measurements using the RC system for 3 days, totaling 6 days in each repetition for five repitions or time points in total. Heifers were housed in temperature-controlled rooms equipped with individual tiestalls (bedded with wood shavings) and provided fresh diets at 0900 h daily. The diet was mainly composed of corn silage, haylage, and grass hay. Heifers had ad libitum access to water and feed and were fed to achieve 5% orts daily. Dry matter intake (DMI) was collected daily throughout the entire experiment. The individual diet components were collected weekly and analyzed using wet chemistry methods (e.g., dry matter, organic matter, aNDFom, CP, sugar, starch, ether extract) and neutral detergent fiber (NDF) digestibility (e.g., at hours 12, 30, 120, and 240) at (Cumberland Valley Analytical Services Inc., Cumberland, MD). Averages ± SD of dietary components over the 5 weeks of collection are outlined in **Table 1**.

Table 1. Chemical composition (mean ± SD) and aNDFom digestibility of forages fed in experiment

Item, % of DM (unless otherwise noted)	Corn silage	Grass hay	Haylage
Dry matter	94.8 ± 0.16	96.5 ± 0.29	93.8 ± 0.41
CP	8.1 ± 0.42	8.7 ± 0.85	15.2 ± 0.35
Soluble protein %CP	64.7 ± 2.51	26.1 ± 2.00	62.7 ± 2.29
ADIPCP	8.3 ± 1.11	20.8 ± 1.57	7.6 ± 1.58
NDIPCP	9.9 ± 0.87	36.6 ± 0.76	11.5 ± 0.96
ADF	20.4 ± 0.80	44.2 ± 0.97	28.3 ± 0.87
aNDFom	34.7 ± 1.29	69.9 ± 1.98	40.4 ± 1.58
Lignin	2.4 ± 0.58	7.6 ± 0.18	3.7 ± 0.37
Starch	36.8 ± 1.72	0.8 ± 1.23	0.3 ± 1.16
Crude fat	4.6 ± 2.13	2.0 ± 2.49	3.5 ± 2.79

ME, Mcal/lb	1.4 ± 0.04	0.9 ± 0.07	1.2 ± 0.02
NDF digestibility, % NDFom			
12h	28.8 ± 5.73	19.7 ± 5.22	49.6 ± 5.60
30h	56.2 ± 6.40	39.9 ± 6.95	68.7 ± 6.58
120h	67.7 ± 3.17	58.5 ± 3.80	76.9 ± 3.97
240h	70.8 ± 3.37	61.1 ± 3.51	79.8 ± 3.64

CP = crude protein, ADIPCP = acid detergent insoluble crude protein, NDIPCP = neutral detergent insoluble crude protein, ADF = acid detergent fiber, aNDFom = amylase treated neutral detergent fiber corrected for organic matter, ME = metabolizable energy, NDF = neutral detergent fiber

For each week of data collection, the feed chemistry of the diet, and herd level DMI and BW were input into CNCPS v.6.5. Using these inputs, methane production was generated at the herd level for each time point. CNCPS generated values were compared to RC values. The comparison between RC and CNCPS was used for total CH_4 predictions including methane production (MP; g/d), and methane yield (MY; g/kg DMI).

Herd average values of BW and DMI along with measurements and predicted values of MP and MY were collected from all heifers over five time points are represented in **Table 2**.

Table 2. Methane production and yield values compared across the five time points with gross percent differences

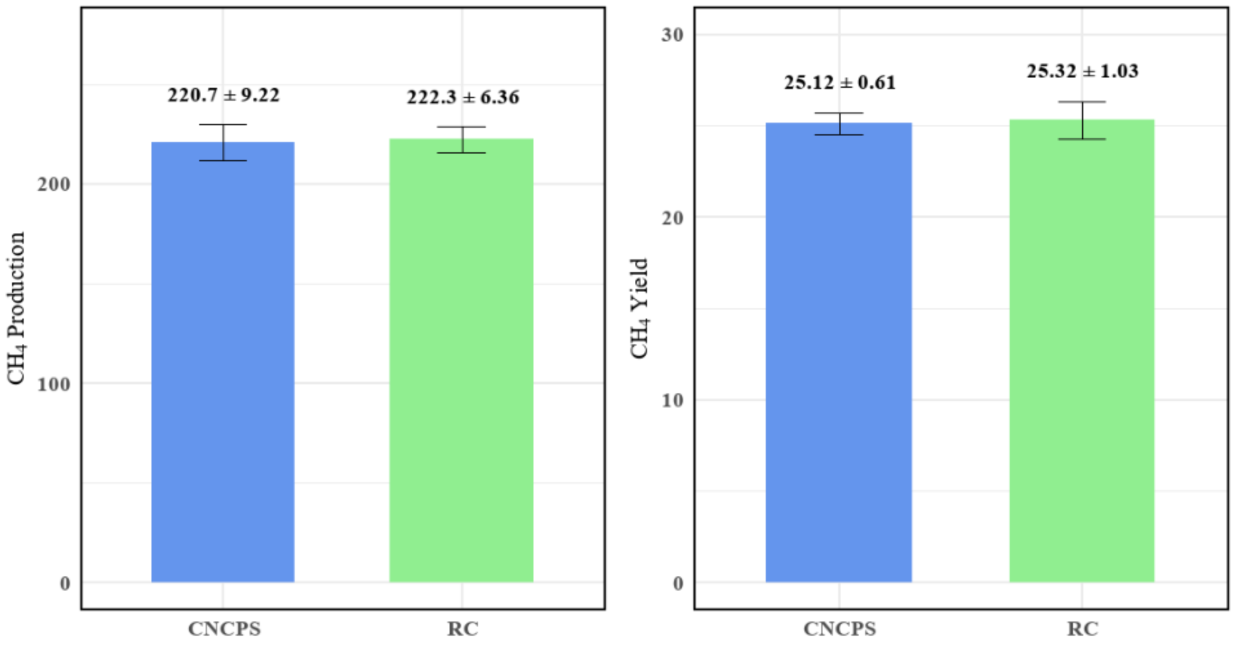
Week	**BW**	**DMI**	**MP (RC)**	**MP (CNCPS)**	**MY (RC)**	**MY (CNCPS)**	**% Difference**
1	567	9.45	227.81	234.34	24.1	24.8	-2.87%
2	571	8.45	224.24	215.07	26.5	25.5	4.09 %
3	574	8.71	224.55	220.15	25.8	25.3	1.96%
4	578	8.68	223.69	224.01	25.8	25.8	-0.144%
5	581	8.67	211.33	210.15	24.4	24.2	0.556%

BW = body weight (kg), DMI = dry matter intake (kg/d), MP = methane production (g CH_4/d), MY = methane yield (g CH_4/kg DMI), RC = respiration chamber, CNCPS = Cornell Net Carbohydrate and Protein System, % Difference = 100 * (Measured MY- Predicted MY) / Measured MY.

Statistical analysis

The MP and MY estimates from RC and CNCPS across the 5 time points were analyzed using R statistical software (Version 4.5.1). A paired two tailed t-test was conducted to evaluate whether the mean difference was zero ($H_0\colon \mu_\Delta = 0;\ H_A\colon \mu_\Delta \neq 0$). Assumptions for the t-test were checked visually using Q-Q plots of mean differences (Δ). As a robustness check that is less sensitive to non-normality, a Wilcoxon signed-rank test was conducted on the same paired differences within MP and MY which tests the null location shift ($\Delta_0 = 0$). The Hodges-Lehmann (HL) estimate of the location shift is interpreted as the difference between RC and CNCPS outputs. Significant differences were declared when $P \leq 0.05$ and tendencies when $0.10 < P > 0.05$. To visualize differences between methods, data were plotted in bar plots with descriptive statstics.

Results and Discussion



Figures 1 and 2. Bar plots illustrating methane production (MP, g CH$_4$/d; left) and methane yield (MY, g CH$_4$/kg DMI; right) values predicted by Cornell Net Carbohydrate Protein System (CNCPS; blue bar) compared to measurements by respiration chambers (RC; green bar). Labeled with mean ± (SD).

The collected methane production data at each of the five timepoints from all eight heifers was averaged within method (CNCPS or RC) as displayed in Figure 1. Across the five time points, the paired t-test found no evidence of a difference in MP between RC and CNCPS (mean difference = 1.58 g/day [RC-CNCPS]; 95% CI [-5.64, 8.79]; t (4) =0.607, *P* = 0.576). To account for methane outputs impacted by intake of the animal, the measured DMI values were factored in to compare methane yield data at each of the five timepoints from all eight heifers averaged within method (CNCPS or RC) as displayed in Figure 2. There was no evidence of a difference in MY between RC and CNCPS (mean difference = 0.20 /day [RC-CNCPS]; 95% CI [-0.62 to 1.02]; t (4) =0.678, *P* = 0.535).

The Wilcoxon signed-rank test conducted on the paired differences similarly was not significantly different between measured and predicted values of methane production (*P* = 0.59) and methane yield (*P* = 0.59). Confidence intervals were not reported due to the small sample size of time points (n = 5) and the discreteness of the signed-rank distribution (*P*-value is provided in Table 3). Wilcoxon signed-rank tests report on the Hodges-Lehmann location shift (HL diff), which was 1.18 for MP and 1.14 for MY, indicating RC values tended slightly higher than CNCPS on average.

Table 3. Statistical comparison of methane production (MP, CH_4 g/d) and methane yield (MY, g CH_4/kg DMI) outputs from respiration chambers (RC) and Cornell Net Carbohydrate Protein System (CNCPS)

Test	**n**	**Statistic**	**df**	**Estimate**	**95% CI**	***P*-value**
MP, CH_4 g/d						
Paired t-test	5	t = 0.607	4	Mean diff = 1.58	[-5.64, 8.79]	0.576
Wilcoxon signed rank	5	V= 10	NA	HL diff = 1.17	NA	0.59
MY, g CH_4/ kg DMI						
Paired t-test	5	t = 0.678	4	Mean diff = 0.20	[-0.62, 1.02]	0.535
Wilcoxon signed rank	5	V = 10	NA	HL diff = 1.14	NA	0.59

df = degrees of freedom, CI = confidence interval, Mean diff = mean of the paired differences, HL diff = Hodges-Lehman location shift, NA = not applicable.

The emissions generated by heifers during the measurement period in the RC reflected the predictive modelling of methane emissions with a $P > 0.05$, thus failing to reject the hypothesis that there was a difference between RC and CNCPS values in this population.

The CNCPS is an accurate tool to quantify baseline enteric methane emissions is already in the hands of many US dairy producers and nutritionists. Looking forward, given the accuracy of the its predictions, CNCPS could be a tool that incorporates methane inhibiting products in the future, and potentially serves as a measurement, monitoring, reporting, and verification mechanism in carbon markets. This may also create an additional source of revenue for farmers to explore with their nutritionists. Examples of this growing market include the GOLD Standard and the Verified Carbon Standard (Environmental Defense Fund, 2024).

Conclusions

An initial step in addressing enteric emissions is to establish robust tools for quantification of emissions for baseline and reductions through interventions. EDF and Cornell University have partnered to analyze the predictive capabilities of CNCPS for quantifying enteric methane emissions. Its functionality for this purpose was reviewed by comparing model-predicted methane outputs with measurements obtained using RC for 8 pregnant Holstein dairy heifers across 5 time points. No significant differences were observed between methane emissions measured in the RC and those predicted by CNCPS, supporting the model's validity as a quantification tool under the conditions of this study. Given that CNCPS has been adopted by the majority of nutritionists in the United States, and considering the model's predictive capacity and consistency with RC measurements, the CNCPS can be an effective and practical tool for the dairy industry to estimate herd-level methane emissions based on diet composition and management practices.

References

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Appendix A: R Code

```
#A comparative analysis of cattle methane emission values measured in respiration chambers vs modeled in CNCPS
library(TOSTER)
library(knitr)
library(showtext)
library(tidyverse)
library(dplyr)
library(gridExtra)
#Data input and table construction
timepoint <-1:5
rc <- c(227.8125, 224.2375, 224.55, 223.6875, 211.3250)
cncps <- c(234.34, 215.07, 220.15, 224.01, 210.15)
bw <- c(567.02, 570.57, 574.11, 577.66, 581.21)
 dmi <- c(9.4463, 8.4472, 8.7095, 8.6752, 8.6661)
 rc_my <- rc/dmi cncps_my <- cncps/dmi
d_mp <- rc- cncps d_my <-rc_my-cncps_my
nmp <- length(d_mp)
nmy <- length(d_my)
Table1 <-data.frame( TimePoint = timepoint, BodyWeight = bw, DMI = dmi, RC_mp= rc, CNCPS_mp = cncps,
    MeanPair_mp = (cncps + rc) / 2, Diff_mp = rc- cncps, RC_my= rc / dmi, CNCPS_my= cncps/dmi, MeanPair_my =
    ((cncps/dmi) + (rc / dmi)) / 2, Diff_my = (rc / dmi) - (cncps/dmi), PctDiff= 100 * (rc- cncps) / cncps )
Table1
#Q-Q Plot
qqnorm(d_mp, main="Q-Q plot of paired differences: Methane Production (g/day)") qqline(d_mp, col=2)
qqnorm(d_my, main="Q-Q plot of paired differences: Methane Yield (g/kg DMI)") qqline(d_my, col=2)
#Outlier visualization
plot(timepoint, d_mp, type="b", pch=19, xlab= "TimePoint",ylab="Difference RC vs CNCPS: Methane Production")
    abline(h=0, lty=2)
plot(timepoint, d_my, type="b", pch=19, xlab= "TimePoint",ylab="Difference RC vs CNCPS: Methane Yield")
    abline(h=0, lty=2)
#t-test and wilcox-signed rank test
Paired t-test (difference ≠ 0)
tt_mp <- t.test(rc, cncps, paired = TRUE, conf.level = 0.95)
wt_mp <-wilcox.test(rc, cncps, paired= TRUE, exact= FALSE)
```

```
 tt_mp

wt_mp

tt_my <-t.test(rc_my, cncps_my, paired = TRUE, conf.level = 0.95)

wt_my <-wilcox.test(rc_my, cncps_my,paired= TRUE, exact= FALSE)

tt_my

wt_my

#barplots

#MP

mp_long <- data.frame( Method = rep(c("RC","CNCPS"), each = n), Value = c(Table1$RC_mp, Table1$CNCPS_mp),
    TimePoint = rep(Table1$TimePoint, times = 2))

summarystats_mp<- mp_long %>% group_by(Method) %>% summarise( n=sum(!is.na(Value)), mean=mean(Value,
    na.rm=TRUE), sd=sd(Value, na.rm=TRUE), se= sd/sqrt(n), .groups="drop" ) summarystats_mp

mp_barplot <- ggplot(summarystats_mp, aes(x = Method, y = mean, fill = Method)) + geom_bar(stat = "identity", width
    = 0.6) + geom_errorbar(aes(ymin = mean - sd, ymax = mean + sd), width = 0.2) + geom_text( aes( label =
    paste0(signif(mean, 4), " ± ", signif(sd, 3)), y = mean + sd + (0.05 * max(mean + sd))
     ), family = "Times New Roman", fontface = "bold", size = 5,
     vjust = 0 ) + scale_fill_manual(values = c("CNCPS"= "#6495ED", "RC"= "#90EE90"))+ theme_minimal() + labs( y
    = expression(CH[4]~Production), x = "")+ theme_minimal(base_family="Times New Roman", base_size=15)+
    theme( text = element_text(family = "Times New Roman", size = 16), axis.title = element_text(face = "plain", size =
    16), axis.text = element_text(face = "bold", size = 14), plot.title = element_text(face = "plain", size = 18, hjust = 0.5))+
    expand_limits(y=275) + theme(legend.position="none")

#MY

my_long <- data.frame( Method = rep(c("RC","CNCPS"), each = n), Value = c(Table1$RC_my, Table1$CNCPS_my),
    TimePoint = rep(Table1$TimePoint, times = 2) )

summarystats_my<- my_long %>% group_by(Method) %>% summarise( n=sum(!is.na(Value)), mean=mean(Value,
    na.rm=TRUE), sd=sd(Value, na.rm=TRUE), se= sd/sqrt(n), .groups="drop" ) summarystats_my

my_barplot <- ggplot(summarystats_my, aes(x = Method, y = mean, fill = Method)) + geom_bar(stat = "identity", width
    = 0.6) + geom_errorbar(aes(ymin = mean - sd, ymax = mean + sd), width = 0.2) + geom_text( aes( label =
    paste0(signif(mean, 4), " ± ", signif(sd, 3)), y = mean + sd + (0.05 * max(mean + sd)) ), family = "Times New Roman",
    fontface = "bold", size = 5,
     vjust = 0 ) + scale_fill_manual(values = c("CNCPS"= "#6495ED", "RC"= "#90EE90"))+ theme_minimal() + labs( y
    = expression(CH[4]~Yield), x = "")+ theme_minimal(base_family="Times New Roman", base_size=15)+ theme( text
    = element_text(family = "Times New Roman", size = 16), axis.title = element_text(face = "plain", size = 16), axis.text
    = element_text(face = "bold", size = 14), plot.title = element_text(face = "plain", size = 18, hjust = 0.5))+
    expand_limits(y=30) + theme(legend.position="none")

mp_barplot <- mp_barplot + theme(panel.border=element_rect(color="black", fill=NA, linewidth=1))

my_barplot <- my_barplot + theme(panel.border=element_rect(color="black", fill=NA, linewidth=1))

grid.arrange(mp_barplot, my_barplot, ncol=2)
```