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Effects of pulse-dosing an essential oil blend to dairy cows on enteric methane emissions and productivity

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ABSTRACT

The objective was to investigate the effects of pulse-dosing the essential oil blend, Agolin Ruminant (EOB), to dairy cows on methane (CH_4) production (g/d) and intensity (g/kg milk or energy-corrected milk (ECM)), as well as lactation performance, on a commercial farm. Seventy-two multiparous, mid-lactation cows were blocked by days in milk, parity, and milk yield and housed in a single pen. After a 2-wk covariate period, cows within each block were randomly assigned to one of two treatments for 10 wk: Control (blank pellets) and EOBP (blank pellets and EOB pellets). The pellets were delivered through double-hopper GreenFeed (GF) units. Hopper 1 of each GF unit contained EOB pellets, formulated to supply ~1 g EOB from the first daily drop of pellets for each EOBP cow. Hopper 2 dispensed blank pellets for the remaining daily drops to EOBP cows and for all drops to Control cows. Enteric gases were measured via the GF units. Milk yields and components were obtained twice weekly, and dry matter intake (DMI) was calculated weekly using milk data, body weight, and body condition score. Overall, cows receiving EOB tended to have lower milk lactose concentration. In week 7, EOBP cows outperformed Control cows in milk yield, ECM, yields of milk protein, lactose, and solids-nonfat, modeled DMI, and CH_4 intensity. Inherent limitations of administering EOB through the GF units, as well as a low number of CH_4 measurements occurring after peak fermentation, may have contributed to the lack of consistent treatment differences. Our efforts to administer a feed additive to individual cows, and our consideration of how farm management practices influenced the results, contribute to the progress of on-farm CH_4 research.

LAY SUMMARY

Cattle contribute to greenhouse gas emissions through enteric methane production. One of the ways to reduce methane emissions is through feed additives. Therefore, the study tested the effects of an essential oil blend, Agolin Ruminant (EOB), on dairy cows to reduce methane emissions and improve milk production. Over 10 wk, the cows receiving EOB did not show differences in methane emissions and milk production except in one week where there was a tendency to have lower methane emissions and greater milk production. Lack of treatment effects could also be due to the method of EOB delivery. This research adds to our understanding of how feed additives can reduce methane emissions on dairy farms.

Key words: dairy farm, enteric methane, essential oils, pulse-dosing.

INTRODUCTION

Recent commitments to reduce greenhouse gas emissions by the global dairy industry (Global Dairy Platform, 2021) and the United States dairy sector (Innovation Center for US Dairy, 2020) require the implementation of scientifically sound on-farm mitigation interventions. Enteric methane (CH_4) is a prime target for reduction as it accounts for 44% of global livestock emissions (GLEAM, 2022) and is a loss of metabolizable energy from ruminants (Johnson and Johnson, 1995). Moreover, rapid and drastic reductions in CH_4 could help slow atmospheric warming due to its short-lived nature and high warming potential (Ocko et al., 2021).

While research into interventions for decreasing enteric CH_4 is actively ongoing (Beauchemin et al., 2022), there are currently few quantifiable options available to farmers. One promising intervention for inhibiting methanogenesis is the provision of feed additives (Honan et al., 2021), such as essential oils (EO). Essential oils are aromatic compounds containing a mixture of plant-secondary metabolites, including terpenoids and phenylpropanoids, with antimicrobial properties capable of modifying rumen fermentation (Busquet et al., 2006; Cobellis et al., 2016).

Agolin Ruminant (Agolin S.A., Bière, Switzerland) (EOB), a commercial EO blend, contains coriander seed oil, eugenol, geranyl acetate, and geraniol. Promising in vitro effects on fermentation and CH_4 have been reported for coriander seed oil (Jahani-Azizabadi et al., 2011), eugenol (Busquet et al., 2006; Joch et al., 2016), and geraniol (Pirondini et al., 2015). Coriander seed oil improved lactation performance in vivo (Matloup et al., 2017), while eugenol did not elicit any effects (Benchaar et al., 2012, 2015). Agolin Ruminant has shown positive yet variable outcomes in vitro (Castro-Montoya et al., 2015; Pirondini et al., 2015) and in vivo (Belanche et al., 2020; Carrazco et al., 2020; Bach et al., 2023). Despite limited research on each component, an undefined mode of action, and inconsistent effects, EOB is one of the few

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potentially anti-methanogenic feed additives with approval for use throughout the United States as a flavoring agent.

On-farm studies play a crucial role in assessing the effectiveness of a feed additive beyond controlled research conditions. However, achieving sufficient statistical power with small effect sizes is challenging on commercial farms. Using pens as experimental units limits sample size (*n*) while delivering treatments to individual cows is often impractical. Consequently, prior on-farm studies involving EOB were conducted on a pen-basis ($n \leq 4$ per treatment) (Santos et al., 2010; Williams et al., 2021; Brambila and Noricumbo-Saenz, 2022; Noricumbo-Saenz et al., 2023) and reported results with either pseudoreplication (denoting "cow" or 'cow within pen' as experimental unit; $n \geq 250$ per treatment) (potentially leading to Type I error) (St-Pierre, 2007; Bello et al., 2016) or insufficient power (potentially resulting in Type II error) (Tempelman, 2009). Although previous studies on commercial farms assessing EOB are valuable, statistically valid on-farm studies with CH_4 measurements are needed.

The primary objective was to investigate the effects of pulse-dosing EOB to individual cows on a commercial farm, delivered through GreenFeed (GF) units (C-Lock, Inc., Rapid City, SD), on CH_4 production (g CH_4/d) and intensity (g CH_4/kg milk or ECM). The secondary objective was to investigate the effects of EOB on lactation performance. It was hypothesized that EOB decreases enteric CH_4 metrics and improves lactation parameters.

MATERIALS AND METHODS

All procedures involving the use of animals were ethically endorsed by the UC Davis Institutional Animal Care and Use Committee (Protocol No. 22740). Data is available at: 10.6084/m9.figshare.26820211.

Animals and Study Design

The study was conducted on a dairy farm in the San Joaquin Valley of California from September to December 2022. The timeline consisted of a 3-wk GF training period, 2-wk covariate, and 10-wk experimental period. Seventy-two multiparous Holstein cows were selected based on DIM (> 90), parity (> 1), milk production (> 36.3 kg), and health status (DairyComp, Valley Agricultural Software). Study cows were housed separately from the rest of the herd in a free-stall pen (< 100% stocking density) with free access to water troughs and the feed bunk. At the start of the trial, the cows averaged 140 ± 24.9 DIM, 2.85 ± 0.929 parity, and 44.1 ± 5.14 kg milk. Cows were blocked by DIM, parity, and milk production, and then randomly assigned to one of two treatment groups: Control (blank pellets only) and EOBP (blank pellets and pellets formulated to provide ~1 g EOB/cow per day). Additionally, each block was assigned to one of two dual-hopper GF units (GFa and GFb; 36 cows per GF); each cow was programmed to receive pellets from one GF to prevent overfeeding in case of lost internet connection. GreenFeeds simultaneously dispense bait and measure gases when prompted by a radio-frequency identification ear tag.

Treatment Administration

The treatments were administered daily via GF units, positioned at opposite ends of the pen. Gates were strategically placed to direct cows into the GF and discourage interruptions. Each GF was equipped with two hoppers: Hopper 1 contained EOB pellets while Hopper 2 contained blank pellets (manufactured by Associated Feed, Turlock, CA). Control cows received blank pellets from Hopper 2 during every visit (Table 1). For EOBP cows, the GF units were programmed to provide EOB pellets from Hopper 1 at the first daily drop, followed by blank pellets from Hopper 2 for the remaining drops throughout the day (Table 1). Data checks were performed nightly to ensure that each cow received the correct hopper configuration from their respective GF. Cows who had not yet visited the GF were encouraged to do so nightly 21:00-23:59h, regardless of treatment (Figure 1). After the study, the visit data were used to determine the timing of EOB consumption relative to TMR delivery, as well as the variability of timing between and within animals.

The inclusion level of EOB in the pellet formulation was determined by dividing the dose of EOB (1 g/cow per day) by the average mass (~38 g) of 40 drops of blank pellets from each GF. The actual daily EOB dose was approximated based on the weekly average mass of 10 researcher-controlled drops from each GF unit and analysis of EOB pellets from Agolin S.A. (Bière, Switzerland) (Supplemental Table S1, 10.6084/m9.figshare.26820580). During the experimental period, the EOB pellets were shorter in length, resulting in a

Table 1. Ingredient and nutrient composition of pellets fed to cows via dual-hopper GreenFeed (GF) units on a commercial dairy farm

Item	Blank Pellets[1]	EOB Pellets[2]
Formulated ingredients[3], %		
Ground wheat	56.1	53.5
Alfalfa meal	40.0	40.0
Wheat millrun	3.72	3.72
Agolin Ruminant (EOB)	—	2.63
Pellet lubricant	0.150	0.150
Formulated nutrients[3], %DM		
CP	15.3	15.0
Crude fiber	11.6	11.5
Ash	6.10	6.00
Crude fat	1.90	1.90
Analyzed nutrients[4]		
DM, %	92.8	92.7
CP, %DM	14.6	14.2
NDF, %DM	25.6	25.2
ADF, %DM	16.0	16.5
TDN, %DM	74.2	75.0
NFC, %DM	53.4	52.5
Starch, %DM	38.3	37.0
Ether extract, %DM	2.65	4.03
Ash, %DM	5.07	5.26
Allowable GF drops/cow, %		
Control cows	100	—
Treatment (EOBP) cows	97.9	2.1

[1]Blank pellets = pellets without Agolin Ruminant, fed through Hopper 2 of GF.
[2]EOB pellets = pellets formulated to contain 2.63% Agolin Ruminant (~1 g/cow per day from one drop of pellets (~38 g of pellets) from Hopper 1 of GF).
[3]Formulated and manufactured by Associated Feed (Turlock, CA).
[4]Analyzed by Cumberland Valley Analytical Services (Waynesboro, PA); $n = 4$.

larger pellet mass and an estimated formulated EOB dose of 1.17 ± 0.085 g/cow per d. However, analysis from Agolin S.A. indicated that the average EOB content was 53.5% lower than formulated, leading to a dose of 0.625 ± 0.0453 g/cow per day.

Samples of pellets were collected four times throughout the study and stored at -20°C until nutrient analyses by Cumberland Valley Analytical Services (CVAS) (Waynesboro, PA) (Table 1). Standard methods (CVAS Inc., 2023) were used to estimate DM%, protein (CP, soluble protein), fiber (ADF, NDF, lignin), ether extract, minerals, ash, as well as calculate NSC and TDN.

Enteric Gases

The GF units measured enteric CH_4, hydrogen (H_2), and carbon dioxide (CO_2) production. Once per week, the GF air filters were changed to maintain adequate airflow (> 27 L/s), and the proximity sensors were cleaned. The GF units auto-calibrated twice weekly (around 04:00h), and additional calibrations were performed within 24 hours before CO_2 recoveries. Recoveries were performed before and after the study, as well as at least once per month. The average CO_2 recovery rate was 101.4% ± 1.15 ($n = 7$) for GFa and 100.1% ± 1.54 ($n = 12$) for GFb. The GF units were programmed to allow a maximum of 6 visits for each cow between 0:00 and 23:59h, with 4 hours allotted between each visit. During each visit, cows were provided a maximum of 8 drops of pellets (average ± SD = GFa: 49.9 ± 4.50 g; GFb: 51.2 ± 5.09 g) every 30s.

Throughout the study, cows had open access to the GF units. In addition, during weeks 6, 8, and 10, cows were encouraged for four days according to the following schedule (once repeated): 08:00-11:59h, 20:00-23:59h, 00:00-01:59h, 12:00-15:59h. The purpose of the additional encouragements was to standardize the timing of measurements across all cows, and the schedule was determined based on the cows' time budget (Figure 1). After the study, gas measurements from GF visits with at least 2 min of continuous breath samples were provided by C-Lock, Inc.

To account for the uneven distribution of visits throughout the day (Figure 1; as demonstrated by Hammond et al. (2015)), weekly enteric gas production was calculated using time bin averaging (Manafiazar et al., 2016). Each enteric gas was averaged weekly within designated time bins (00:00:00–03:59:59h, 04:00:00–07:59:59h, 08:00:00–11:59:59h, 12:00:00–15:59:59h, 16:00:00–19:59:59h, and 20:00:00–23:59:59h). The resulting six values were then averaged to obtain one weekly value. For inclusion in the final dataset, a weekly value needed to have measurements from at least two time bins. The variability in CH_4 production measurements was calculated using within- and between-cow coefficients of variation (CV) (C-Lock Inc., 2020).

Milk Yield and Components

Milk yields and component samples (≤ 60 mL) were collected two days per week during two consecutive milking shifts (~06:30h and ~18:30h) using Tru-Test meters from Central Counties DHIA (Atwater, CA). The samples were preserved in 2-bromo-2-nitropropane-1,3-diol until analysis for fat (%), true protein (%), solids nonfat (SNF) (%), lactose (%), milk urea nitrogen (mg/dL), and somatic cell count (SCC) (10^3 cells/mL) (Central Counties DHIA, Atwater, CA).

Biologically unrealistic data, including 1 milk yield, 4 components, and 1 milk urea nitrogen value, were removed from the dataset. Two SCC values were excluded due to mastitis in one cow during the covariate. Outliers were identified in the morning and evening measurements for each cow using three SD from the mean and removed if deemed necessary (5 milk yields and 1 SCC). The milk yields and component concentrations were averaged for AM and PM milking shifts each week and used to calculate the weekly weighted milk component concentrations (%) and yields (kg). The weekly milk yields were the sum of the averaged AM and PM shifts. The resulting component and milk yields were then used to calculate ECM

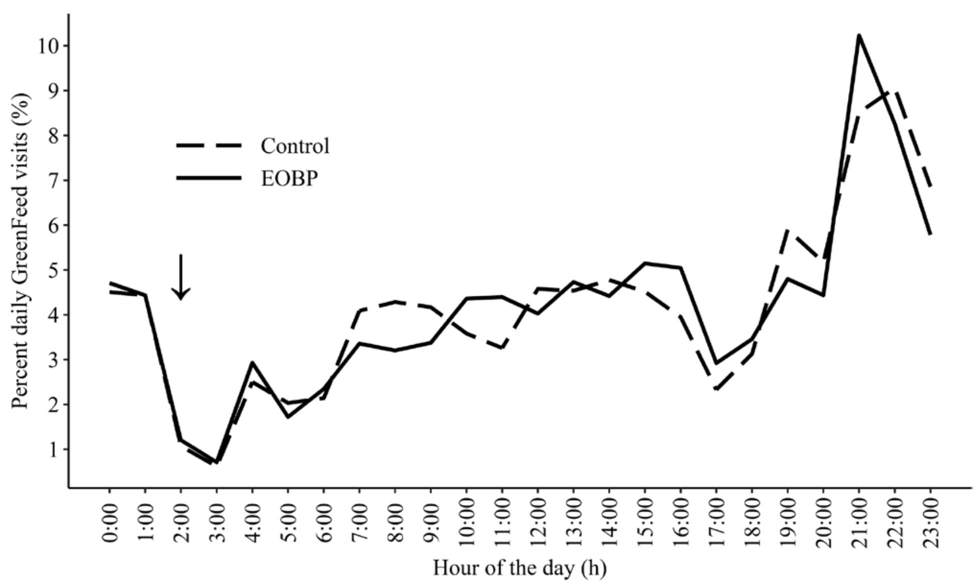

Figure 1. Visits and Time Budget of Study Cows. Diurnal pattern of GreenFeed (GF) visits (expressed as percent of total daily visits), along with approximate time budget of the study cows. Arrow indicates typical timing of feed delivery. Treatments: Control = blank pellets; EOBP = blank pellets + pellets formulated to provide ~1 g Agolin Ruminant/cow per day.

using the equation adapted from Tyrrell and Reid (1965): $ECM(kg) = 0.327 \times \text{milk yield (kg)} + 12.95 \times \text{milk fat (kg)} + 7.65 \times \text{milk true protein (kg)}$. Finally, the CH_4, CO_2, and H_2 intensities (g/kg milk and ECM) were calculated.

Body Condition Score, Body Weight, and Lameness

Body condition was evaluated weekly using a 5-point scale and averaged across the same three scorers. Body weight was measured weekly by the same researcher between 02:00-04:00h using a weight tape (Weigh-By-Breed Dairy Tape, The Coburn Company, Inc., Whitewater, WI). Three unrealistic BW were removed prior to analysis. Any lame animals were reported to the farm for inclusion in the herd's weekly hoof trimming. Lameness prevalence was calculated based on researcher observations and DairyComp records.

Rumen Fluid pH

Rumen fluid samples were collected (Control: $n = 6$; EOBP, $n = 6$) during the covariate, and weeks 3, 6, and 9, between 10:00h and 12:00h, using the Drench-Mate Cow Rumen Fluid Extractor (Drench-Mate, Sumas, WA). After the rumen fluid was filtered through two layers of cheesecloth into a glass beaker, the pH was recorded using a calibrated pH meter (Extech PH100 ExStik pH Waterproof Meter). Three 1.5 mL aliquots of each sample were stored in 2 mL cryovials at – 20°C until volatile fatty acid (VFA) analysis by the UC Davis Hess Laboratory (Davis, CA).

Estimated Dry Matter Intake

Cows were fed a TMR once daily at ~02:00h, and feed was pushed up at the bunk multiple times per day. The formulated nutrient and ingredient compositions of the TMR, as provided by the nutritionist, are presented in Supplemental Table S2 (10.6084/m9.figshare.26820580). The study intended ad libitum intake, but the feed bunk was often observed as empty or sorted by the evening milking.

Individual DMI was calculated using the animal parameter equation from NASEM (2021):

$$\begin{aligned}\text{DMI (kg)} &= [(3.7 + \text{parity} \times 5.7) + 0.305 \times \text{milk energy (Mcal)} + 0.022 \times \text{BW (kg)} + (-0.689 + \text{parity} \times -1.87) \\ &\times \text{BCS}] \times [1 - (0.212 + \text{parity} \times 0.136) \times \exp(-0.053 \times \text{DIM})].\end{aligned}$$

Milk energy (NASEM, 2021) was adjusted for the monohydrate form of lactose (Hall, 2023):

$$\begin{aligned}\text{Milk energy (Mcal)} &= \text{milk yield (kg)} \times (0.0929 \times \text{milk fat }\% \\ &+ 0.0563 \times \text{milk true protein }\% \\ &+ 0.0376 \times \text{milk lactose }\%).\end{aligned}$$

To check the accuracy of the modeled individual DMI, pen DMI was measured weekly. Offered and refused TMR samples were collected once per week and frozen at – 20°C until analysis by CVAS for DM%. The DM percentages were multiplied by the as-fed values extracted from FeedWatch (Valley Agricultural Software) to calculate pen DMI, which was then divided by the number of cows in the pen. Every week, the average modeled DMI was within 4 kg of pen DMI/cow numbers.

Statistical Analysis

The experimental unit was the individual cow because the treatment was applied independently to each animal, and all cows were housed together in a single pen. The required n per treatment was determined using a balanced one-way ANOVA power calculation considering the expected reduction in CH_4 production. With two groups, a between-variation of 10%, within-variation of 36, a significance level of 0.05, and a power of 0.80, 30 cows per treatment were needed. To account for potential health and GF visit issues, 36 animals per treatment were enrolled. Four cows were removed from data analysis due to lameness concerns that prevented them from completing the study (2 Control and 2 EOBP), and one EOBP cow was removed due to low outlying milk data and digestive issues. The final datasets consisted of 67 cows (Control: $n = 34$ and EOBP: $n = 33$).

Data collected while cows were in the hospital pen were omitted. This included two Control cows for most of the covariate period and one EOBP cow for most of weeks 6 to 9 (EOB pellets were top-dressed daily to the cow during this time).

Weekly averages were analyzed using the statistical software R (v4.2.2, R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were analyzed using a linear mixed effects model (nlme::lme) with treatment as the fixed effect and treatment within block as the random effect. Outliers were removed from the experimental period using the interquartile range method, with less than 5% of data removed. Experimental data were analyzed with a linear mixed effects model with a first-order autoregressive correlation structure, considering the fixed effects of the covariate, treatment, week, and interaction between treatment and week, along with the random effect of treatment within block. During model selection for the enteric gases, the assigned GF was removed due to lack of significance.

After model selection for the experimental data, normality of the standardized residuals was confirmed through quantile-quantile plots and the Shapiro-Wilkes test. Homogeneity of variance was assessed on the standardized residuals using residuals vs fitted plots. To meet the assumption of normality, SCC was transformed with a binary logarithm, and results were converted back to interpretable values. Overall least square means (LSM) ± standard error (SE) were determined via a pairwise comparison test (emmeans::emmeans) with ANOVA p-values. Significance for all datasets was declared as $P < 0.05$, with trends set at $0.05 \leq P \leq 0.10$. Whenever a treatment × week interaction demonstrated a significance or trend, the parameter was graphed weekly with pairwise comparison LSM and standard error of the means (SEM).

RESULTS AND DISCUSSION

To our knowledge, this study was the first attempt at using dual-hopper GF for administering treatments to dairy cows, thereby enabling assessment of EOB in a commercial setting free from the constraints of insufficient or inflated statistical power. Beyond the stated objectives, the novelty of our methods and the limitations inherent of this study raise important considerations for the future: 1) for subsequent studies employing similar methods and 2) for understanding the role of farm management in assessing the effectiveness of anti-methanogenic feed additives.

Enteric Gases

Our finding of no effect on CH_4, CO_2, and H_2 production (Table 2) aligns with two other pulse-dose studies, one of which used EOB (Carrazco et al., 2020) and another tested a similar product (eugenol and geranyl acetate blend) (Silvestre et al., 2023). Similarly, EOB did not affect CH_4 production in Holstein beef steers when included in the ration (Miller et al., 2023). In our study, the acetate-to-propionate ratio also did not differ between treatments from a subset of cows (Supplemental Table S3, 10.6084/m9.figshare.26820580). However, the literature generally reports a decrease in CH_4 production from EOB, but the nature of the reduction is inconsistent. One notable finding was an immediate and sustained drop when EOB was included in a TMR containing no silages and with CH_4 captured twice daily after milkings (Bach et al., 2023). Methane reductions from EOB were also reported by Klop et al. (2017a,b), but the effects were transient within five weeks. However, Klop et al. (2017a) lacked an unsupplemented group of animals, and Klop et al. (2017b) reported CH_4 from in vitro measurements. In contrast to immediate reductions, a 4-week adaptation period was suggested by a meta-analysis on EOB (Belanche et al., 2020). Results from the meta-analysis must be considered cautiously as 4 of the 8 CH_4 studies were not published in a peer-reviewed journal, 2 studies measured CH_4 using in vitro methods (Klop et al., 2017b; Elcoso et al., 2019), 1 reported results with psuedoreplication (Hart et al., 2019), and 1 used the pretreatment period as the control, resulting in a confounding effect of time (Castro-Montoya et al., 2015).

In our study, the cows' time budget (Figure 1) may have influenced enteric gas results. Locking cows at the feed bunk is common for disease and reproductive management, but rumination conditions after feeding were likely suboptimal due to standing in headlocks ~02:00-04:00h and visiting the parlor ~05:30-07:30h (Cooper et al., 2007; Schirmann et al., 2012). Also, headlocks hindered the ability to accurately capture CH_4 directly postprandially and limited total weekly visits (< 20) (Table 3). Although it is speculated that fewer weekly visits (≥ 12) are necessary with sufficient statistical power (Jonker et al., 2020), the average first gas measurement relative to feed delivery for both Control (9.7 ± 6.1 h) and EOBP (10.2 ± 6.2 h) occurred after peak CH_4 emissions (Crompton et al., 2011) when potential treatment differences may have been more pronounced.

Similar to CH_4 production, effects of EOB on CH_4 intensity are inconsistent in the literature. In line with our findings of no overall treatment effects, Castro-Montoya et al. (2015) and Bach et al. (2023) reported no differences in CH_4 intensity. In contrast, others found that EOB decreased CH_4 intensity on an ECM basis (Klop et al., 2017a; Hart et al., 2019; Carrazco et al., 2020). In week 7 of our study, CH_4 intensity was 9.70% lower in the EOBP group (8.75 g/kg milk vs. 9.69 g/kg milk; SED = 0.318; $P = 0.006$; Figure 2), resulting in a tendency for a treatment×week interaction ($P = 0.071$; Table 2). The lower CH_4 intensity in week 7 was driven by a numerical decrease in CH_4 production and a tendency for greater milk yield in EOBP cows. The methane intensity effect on week 7 must be interpreted with caution. Since there was only a tendency for week×treatment interaction and the effect was only observed on 1 week out of 10, it could be due to variation in the methane and milk measurements during that week.

Lactation Performance

No overall treatment differences were detected in lactation performance (Table 4) besides a tendency for lower milk lactose concentration in EOBP cows ($P = 0.078$). In contrast, Silvestre et al. (2023) reported an increase in lactose % from a eugenol and geranyl acetate blend. Our finding of no effect on milk yield, components, and ECM aligns with others that fed EOB to individual cows (Klop et al., 2017b; Carrazco et al., 2020; Bach et al., 2023). However, our results conflict with Hart et al. (2019) and Noricumbo-Saenz et al. (2023), both of which had pseudoreplication. No differences in BW and BCS suggest that EOB does not alter metabolism, contrasting with

Table 2. Effect of pulse-dosing Agolin Ruminant (EOB) through GreenFeed (GF) units over 10 weeks on enteric gas emissions from dairy cows on a commercial farm

	Treatment[1]			p-value[2]		
Item	Control	EOBP	SED	T	W	T × W
CH_4, g/d	329 ± 4.4	326 ± 4.4	6.0	0.597	<0.001	0.199
CH_4, g/kg milk	8.74 ± 0.158	8.49 ± 0.159	0.202	0.267	<0.001	0.071
CH_4, g/kg ECM[4]	7.74 ± 0.134	7.50 ± 0.134	0.161	0.159	<0.001	0.134
CO_2, g/d	12300 ± 100	12400 ± 100	131	0.491	<0.001	0.192
CO_2, g/kg milk	325 ± 4.8	323 ± 4.8	6.2	0.805	<0.001	0.259
CO_2, g/kg ECM[4]	290 ± 3.8	285 ± 3.8	4.9	0.349	<0.001	0.110
H_2, g/d	1.59 ± 0.048	1.63 ± 0.048	0.061	0.488	<0.001	0.730
H_2, g/kg milk	0.042 ± 0.0014	0.042 ± 0.0014	0.0018	0.992	<0.001	0.591
H_2, g/kg ECM[4]	0.037 ± 0.0012	0.037 ± 0.0012	0.0015	0.951	<0.001	0.798

[1]Treatments: Control=blank pellets; EOBP=blank pellets + pellets formulated provide ~1g EOB/cow per day; values expressed as least square means ± standard error of the mean.
[2]T=Treatment; W=Week; T × W=Treatment × Week.
[4]ECM (kg)=0.327 × milk kg + 12.95 × milk fat kg + 7.65 × milk true protein kg; adapted from Tyrrell and Reid (1965).
Gas values based on average of measurements in a week from GF visits occurring in ≥ 2 time bins. The time bins are as follows: 00:00–03:59, 04:00–07:59, 08:00–11:59, 12:00–15:59, 16:00–19:59, and 20:00–23:59.

an on-farm study (Santos et al., 2010) that indicated potential body fat mobilization.

Despite no overall treatment differences, there was an interaction between treatment and week (Table 4) for milk yield ($P = 0.061$), ECM ($P = 0.023$), fat yield ($P = 0.025$), protein yield ($P = 0.086$), lactose yield ($P = 0.070$), SNF yield ($P = 0.081$), and modeled DMI ($P = 0.031$). Although the covariate measurements were included in the model, the lower milk fat yield ($P = 0.050$; Supplemental Figure S1, 10.6084/m9.figshare.26820580) and ECM ($P = 0.045$; Figure 3) in EOBP cows in week 1 can be partially explained by the descriptive statistics (Supplemental Table S4, 10.6084/m9.figshare.26820580), which show a significance ($P = 0.019$) and trend ($P = 0.089$) in the respective parameters. The treatment × week interaction in milk yield was driven by a tendency in week 7 for EOBP cows to produce more than Control cows ($P = 0.052$; Figure 3). Week 7 also had a trend for increased protein ($P = 0.053$), lactose ($P = 0.078$), and SNF yields ($P = 0.069$) (Supplemental Figure S1, 10.6084/m9.figshare.26820580), as well as ECM ($P = 0.051$; Figure

Table 3. GreenFeed (GF) visit information and methane (CH_4) variability by treatment

Item	Treatment[1]	
	Control	EOBP
Total visits		
Covariate	730	749
Experimental	4774	4636
Weekly n[2]	32.6 ± 0.505	32.3 ± 0.467
Cows present in all weeks	31	31
Weekly visits per cow[2]	15.3 ± 2.47	15.1 ± 2.84
% cows with ≥ 20 visits[2,3]	16.8 ± 6.51	16.4 ± 10.15
Duration of visits[2]	3:59 ± 0:00:27	4:01 ± 0:00:28
CH_4 CV, %		
Between Cow	10.9	13.7
Within Cow	10.9	11.6

[1]Treatments: Control=blank pellets; EOBP=blank pellets + pellets formulated to provide ~1g Agolin Ruminant/cow per day from the GF.
[2]Values expressed as mean ± SD.
[3]Average percent of cows per week with 20 or more visits.

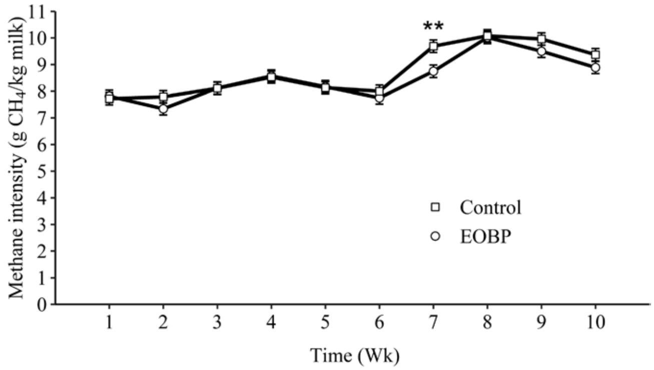

Figure 2. Methane Intensity. Methane (CH_4) intensity (g/kg milk) on a commercial dairy farm over ten weeks between two treatments: Control (blank pellets) and EOBP (blank pellets + pellets formulated to provide ~1 g Agolin Ruminant/cow per day). Points are covariate-adjusted least-square means and bars are standard error of the mean. In wk 7, EOBP had lower CH_4 intensity than Control (8.75 g/kg vs. 9.69 g/kg; SED = 0.318; $P = 0.006$).

Table 4. Effect of pulse-dosing Agolin Ruminant (EOB) through GreenFeed (GF) units over 10 weeks on milk yield and components, BW, BCS, and modeled DMI for dairy cows on a commercial farm

Item	Treatment[1]			p-value[2]		
	Control	EOBP	SED	T	W	T × W
Milk yield, kg/d	38.5 ± 0.46	39.1 ± 0.47	0.56	0.456	<0.001	0.061
ECM[3], kg/d	43.1 ± 0.48	43.7 ± 0.48	0.54	0.337	<0.001	0.023
Fat, %	4.10 ± 0.039	4.17 ± 0.040	0.057	0.295	<0.001	0.603
Fat, kg/d	1.57 ± 0.019	1.59 ± 0.019	0.023	0.363	0.004	0.025
Protein, %	3.44 ± 0.013	3.44 ± 0.014	0.019	0.867	<0.001	0.943
Protein, kg/d	1.32 ± 0.015	1.34 ± 0.016	0.018	0.357	<0.001	0.086
Lactose, %	4.83 ± 0.009	4.81 ± 0.009	0.009	0.078	<0.001	0.821
Lactose, kg/d	1.86 ± 0.024	1.88 ± 0.024	0.028	0.556	<0.001	0.070
SNF, %	9.20 ± 0.018	9.16 ± 0.018	0.023	0.114	<0.001	0.990
SNF, kg/d	3.54 ± 0.043	3.58 ± 0.044	0.050	0.527	<0.001	0.081
MUN, mg/dL	11.0 ± 0.11	11.2 ± 0.11	0.12	0.107	<0.001	0.427
SCC[4],10^3 cells/mL	79.3 ± 1.10	76.1 ± 1.10	1.13	0.647	<0.001	0.983
BW, kg/d	716 ± 2.5	718 ± 2.6	3.6	0.583	<0.001	0.727
BCS	2.82 ± 0.022	2.82 ± 0.022	0.029	0.837	<0.001	0.334
mDMI[5], kg/d	26.7 ± 0.14	26.9 ± 0.14	0.18	0.200	<0.001	0.031

[1]Treatments: Control=blank pellets; EOBP=blank pellets + pellets formulated provide ~1g Agolin Ruminant/cow per day; values expressed as least square means ± standard error of the mean.
[2]T=Treatment; W=Week; T × W=Treatment × Week.
[3]ECM (kg) = 0.327 × milk kg + 12.95 × milk fat kg + 7.65 × milk true protein kg; adapted from Tyrrell and Reid (1965).
[4]Log-transformed data used for statistical analysis.
[5]Modeled DMI calculated according to NASEM (2021).

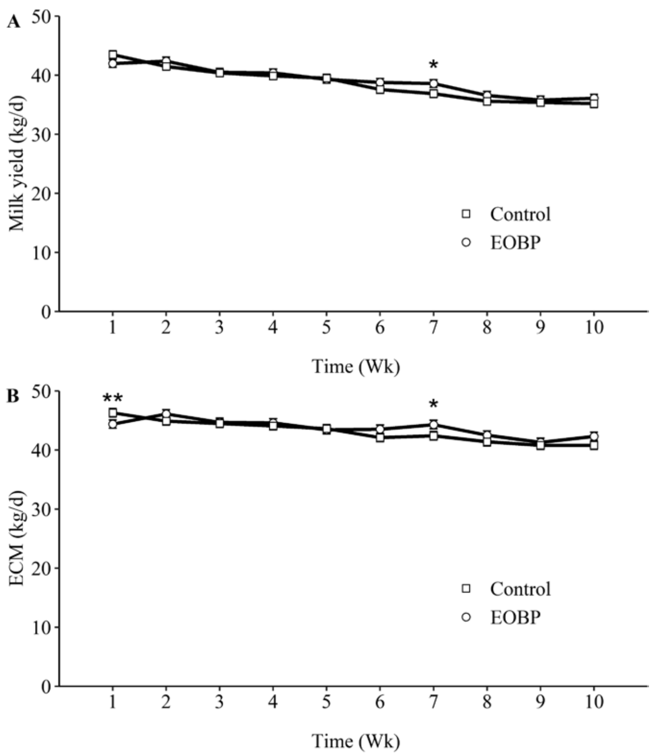

Figure 3. Milk Yield and ECM. A) Milk yield (kg/d) and B) ECM (kg/d) on a commercial dairy farm over ten weeks between two treatments: Control (blank pellets) and EOBP (blank pellets + pellets formulated to provide ~1 g Agolin Ruminant/cow per day). Points are covariate-adjusted least-square means, and bars are standard error of the mean. In wk 7, EOBP tended to have greater milk yield than Control (38.6 kg vs. 36.9 kg; SED = 0.84; P = 0.052). Cows in the EOBP treatment had lower ECM than Control in wk 1 (44.4 kg vs. 46.3 kg; SED = 0.90; P = 0.045) and tended to have greater ECM in wk 7 (44.3 kg vs. 42.4 kg; SED = 0.91; P = 0.051).

3) and modeled DMI ($P = 0.037$; Supplemental Figure S2, 10.6084/m9.figshare.26820580) from EOB.

Similar to our study, Elcoso et al. (2019) fed EOB to individual animals separately from the TMR, reporting increased milk and component yields in select weeks. Unlike our results, Elcoso et al. (2019) observed consistently greater ECM during the last weeks of supplementation, indicating sustained improvements from EOB. Our observation of improved milk yield and ECM exclusively in week 7 is more challenging to interpret. During week 7, there was a decrease in GF visits, likely due to a new batch of pellets. Additionally, in the preceding week, encouragement to the GF was provided throughout the day, which briefly disrupted time budgets. Therefore, it is plausible that the higher milk yield in week 7 can be attributed to increased lying time, coupled with the fresh batch of EOB pellets. It could be argued that EOB reached its impact potential in week 7, and the rumen adapted in subsequent weeks.

Treatment Administration Method

Innovative methods for administering treatments advance on-farm research. In a pasture-based beef operation, Beck et al. (2021) reported similar results when comparing hand-fed and GF-fed methods, showing promise for GF as an effective treatment administration method. However, a limitation is the lack of control and knowledge of the treatment dosage, partially attributed to the voluntary nature of cows visiting the GF. Although complete consumption of the pellets cannot be guaranteed, all cows in our study were recorded as receiving their treatment (except for one cow for 2 d in wk 9). However, nightly GF encouragement for cows who had not yet received their treatment (averaging 6.1 ± 2.70 cows per night) clumped enteric CH_4 measurements and promoted EOB pellet consumption approximately 20 h after feed delivery. Additionally, on average, EOB consumption occurred 11.0 ± 8.07 h post-feed delivery, past the peak of fermentation. A high variation in the timing of EOB consumption between (CV = 19.8%) and within (CV = 72.4%) animals could have influenced fermentation patterns. As EOB was administered through GF units, fluctuations in intake timing may have affected rumen microbial activity, fermentation dynamics, and ultimately CH_4 emissions. It should also be noted the estimated EOB dose in the pellets was lower than intended. In future experiments, dosage analysis should be conducted weekly to ensure the intended dose is consistently administered. Conducting controlled experiments in a facility capable of measuring intake from a large number of animals would facilitate EOB administration with feed, reducing variability. Our attempt at using GF for treatment delivery contributes to the exploration of methods to improve on-farm research. An alternative is to deliver treatments via RFID-controlled feed bins that also provide daily TMR allowance, a system which is often used in controlled research and some on-farm studies, allowing for the measurement of intake from a large number of animals and more consistent EOB consumption.

Implications of Potential Acidosis

The cows appeared to be susceptible to acidosis due to a combination of risk factors, including a low BCS (Table 4), high lameness prevalence (Control: 36%; EOBP: 31%), loose manure, and feed consumption from a single, large meal. While the mean rumen pH values from a subset of cows were within an acceptable range (Control: 6.79 ± 0.487; EOBP: 6.82 ± 0.336), the daily bouts of low pH expected with acidosis may not have been captured (Jonsson et al., 2019). Plant secondary compounds have been considered a management intervention for reducing the risk of acidosis (Humer et al., 2018; Elmhadi et al., 2022) whereas others have found that EO may worsen the condition (Yadeghari et al., 2015; da Silva et al., 2020). Nevertheless, numerically lower lameness and higher rumen pH in EOBP cows warrant further EOB research in acidotic cows.

CONCLUSIONS

Agolin Ruminant had no overall effects on CH_4 parameters but lowered CH_4 intensity by 9.70% in week 7, driven by a tendency for increased milk yield and numerically lower CH_4 production. Besides an overall tendency for low milk lactose concentration as well as low ECM and milk fat (kg) in week 1, EOB did not negatively affect lactation performance. The limitations included the time budget of the cows and the treatment administration method, both of which lend themselves to important discussion for the improvement of on-farm antimethanogenic research.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. JMT is employed by the funding organization.

Author Contributions

Julia Quinn Fouts (Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft), Silvia Grossi (Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - review & editing), Juan M. Tricarico (Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing - review & editing), and Ermias Kebreab (Conceptualization, Funding acquisition, Resources, Supervision, Validation, Writing - review & editing)

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