



# Replacement of Soybean Meal With Soybean Cake Reduces Methane Emissions in Dairy Cows and an Assessment of a Face-Mask Technique for Methane Measurement

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The objective of this study was to (a) evaluate the effect of replacing soybean meal (SBM) with soybean cake (SBC) on feeding behavior, rumen fermentation, milk production, nutrient digestibility and CH<sub>4</sub> emissions and (b) investigate whether a face-mask technique could be used to predict daily methane (CH<sub>4</sub>) emissions in dairy cattle. The experiment was conducted as a completely randomized design, with 32 crossbred Holstein × Gyr cows (days in milk (DIM): 112 ± 25.1) randomly assigned to the following treatments (*n* = 8/group) for 75 days: (1) 0% SBC, (2) 6% SBC, (3) 14% SBC, and (4) 23% SBC, in place of SBM on a dry matter (DM) basis. Across the final 4 weeks of the study, CH<sub>4</sub> production was estimated using the proposed face-mask technique subsequent to a respiration chamber measurement for an evaluation of treatment efficacy and face-mask accuracy. There was no effect of SBM replacement by SBC on intake, feeding or drinking behavior (*P* > 0.21). Total VFA concentration, the individual proportions of VFA and blood metabolites were not altered (*P* > 0.17) by SBC, however there was a tendency for decreased (*P* = 0.08) lactate and plasma urea nitrogen (*P* = 0.07) concentration associated with SBC addition. Fat-corrected milk yield (FCM<sub>4%</sub>) and composition was not affected (*P* > 0.27) by SBC; however, there was a tendency for decreased total milk solids (*P* = 0.07) and milk fat (*P* = 0.08) associated with 23% SBC treatment. There was no treatment × technique interaction (*P* > 0.05) effect on gas measurements. A maximum reduction (*P* = 0.01) in CH<sub>4</sub> yield (g/kg DM) and intensity (g/kg milk) of 11 and 20%, respectively, was observed for the 14% SBC inclusion. Compared to the week of mask measurements, chambers decreased (*P* = 0.01) intake (kg/d, %BW) and increased (*P* = 0.05) FCM<sub>4%</sub>. The face-mask method over estimated O<sub>2</sub> consumption by 5%. The

face-mask method accurately predicted daily CH<sub>4</sub> emissions when compared to the chamber at the same time-point. However, there was a linear bias of CH<sub>4</sub> outputs so further evaluation of the calculation of total CH<sub>4</sub> from a spot measurement is required.

**Keywords:** co-product, dairy cattle, greenhouse gas, climate change, respiration chamber

## INTRODUCTION

The expansion of the biofuel industry has contributed, in part, toward a rise in livestock grain and oilseed prices. As a result, the utilization of co-products derived from biofuel manufacturing is of increasing popularity as a low cost feed alternative (1). Due to the high fat content of biofuel co-products (2–4), there is a potential for the utilization of these feedstuffs as a methane (CH<sub>4</sub>) mitigation tool. Dietary fats have been proven to decrease CH<sub>4</sub> emissions through the suppression of methanogen and ciliate protozoa populations, dilution through replacement of fermentable carbohydrates, reduction of ruminal organic matter fermentation and biohydrogenation of free unsaturated fatty acids (5–7). Dried distillers grain originating from bioethanol production have been proven to decrease enteric CH<sub>4</sub> emissions (4, 8), however, more research efforts are needed to investigate the mitigating properties of biodiesel co-products. As the desire for the use of all biofuel co-products in livestock production increases, an investigation into biodiesel co-products as a mitigation strategy is of significance to livestock industries worldwide.

Soybeans are one of the most common feedstock for biodiesel manufacturing globally (9). The resultant high fat co-product, referred to as soybean cake (SBC), obtained by a physic process (pressure and heat) of oil extraction, is an available co-product and possibly an alternative to soybean meal (SBM). The effective replacement of SBM with SBC in livestock diets, in addition to the potential mitigating properties of this feed source, has wide-reaching economic and environmental importance. Additionally, SBC may be an option for the organic dairy market, which requires milk production to be free of chemicals.

A need for inexpensive and convenient methods in the estimation of livestock CH<sub>4</sub> emissions has been identified. Currently, the respiration chamber is considered the gold standard method for quantification of enteric CH<sub>4</sub> emissions (10). However, chamber use is limited on a practical research basis and cannot be implemented on farm due to costly infrastructure, lengthy observation periods, and alterations to animal behavior (10–12).

The investigation of alternative CH<sub>4</sub> measurement techniques in recent studies has focused primarily on the calculation of daily emissions from spot samples of eructated and respired air. Garnworthy et al. (11) and Huhtanen et al. (10) accurately predicted daily CH<sub>4</sub> emissions based on samples obtained via specialized feeders. Based on these outcomes, and utilizing existing chamber infrastructure, an updated face-mask method involving the measurement of respired and eructated air has been proposed as a more practical means by which total daily emissions can be quantified (13). Previous use of the

face-mask technique involved 30 min measurement periods taken every 2–3 h throughout the day (14). The high frequency of measurements were found to lead to altered animal behavior and therefore did not represent actual emissions. Due to the strong correlation between total daily emissions and spot samples at specific times post feeding, the number of samples taken throughout the day could be reduced, minimizing the impact on animal behavior.

The objective of this study was to (a) evaluate the effect of replacing soybean meal (SBM) with soybean cake (SBC) on feeding behavior, rumen fermentation, milk production, nutrient digestibility, and CH<sub>4</sub> emissions and (b) assess whether the proposed face-mask method could accurately predict daily CH<sub>4</sub> emissions when compared to the gold standard respiration chamber. It was hypothesized that the SBC would reduce CH<sub>4</sub> emissions in crossbred dairy cows, without impacting animal intake or performance and that the face-mask method could accurately predict daily CH<sub>4</sub> production.

## MATERIALS AND METHODS

The experiment was conducted in the Bioenergetics Laboratory of the Brazilian Agricultural Research Corporation (EMBRAPA), at the Multi-use Complex on Livestock Bioefficiency and Sustainability at Embrapa Dairy Cattle, in Coronel Pacheco, Minas Gerais, Brazil. All animal care and handling procedures were approved by the Embrapa Dairy Cattle Animal Care and Use Committee (Juiz de Fora, Minas Gerais, Brazil; Protocol No. 28/2014).

### Animals, Experimental Design, and Treatments

Thirty-two lactating crossbred cows (5/8 Holstein × Gyr) were selected based on days in milk (DIM; 112 ± 25.1), milk yield (20.8 ± 2.92 kg/d), and body weight (BW; 551 ± 45.4). Animals were randomly assigned to four dietary treatments (*n* = 8 per treatment) over 75 days. The first 21 days consisted of a dietary adaptation, followed by 54 days measurement period. All cows were exposed to both the chamber and face-mask across the final 28 days of the measurement period. The dietary treatments consisted of (1) 0% SBC (control diet; CON), (2) 6% SBC (6% SBC), (3) 14% SBC (14% SBC), and (4) 23% SBC (23% SBC) on a DM basis. Cows were housed in a covered freestall for the experimental period, except during milking, and periods of methane measurement using the respiration chambers and face-mask. Respiration chambers and the face-mask technique were conducted within a controlled environment facility.

**TABLE 1** | Ingredients and chemical composition of diets.

Item	Treatment			
	Control	6% SBC	14% SBC	23% SBC
<b>Ingredients (% DM)</b>				
Corn silage	49.2	49.2	49.2	49.2
Tifton hay	5.4	5.4	5.4	5.4
Corn grain, fine ground	22.2	21.9	21.6	21.3
Soybean meal <sup>a</sup>	22.2	16.5	8.6	0.0
Salt	0.3	0.3	0.3	0.3
Soybean cake (SBC) <sup>b</sup>	0.0	5.9	14.1	23.0
Limestone	0.7	0.7	0.7	0.7
Mineral supplement	0.2	0.2	0.2	0.2
<b>Chemical composition</b>				
Dry matter (%)	43.3	43.3	43.2	43.2
Crude protein (CP, % DM)	17.3	17.3	17.3	17.3
Neutral detergent fiber (NDF, % DM)	29.4	27.9	29.2	28.9
NFC <sup>c</sup> (% DM)	44.5	46.4	43.9	43.1
Ether extract (EE, % DM)	3.1	3.6	4.2	4.9
Organic matter (% DM)	94.3	95.2	94.6	94.2

Control, no SBC; 6% SBC, 6% DM of soybean cake replacing soybean meal; 14% SBC, 14% DM of soybean cake replacing soybean meal; 23% SBC, 23% DM of soybean cake replacing soybean meal.

<sup>a</sup>Nutrient content of soybean meal composition (% in the DM): 48.8 CP, 14.6 NDF, 28.3 NFC, 1.7 EE, 6.6 ash.

<sup>b</sup>Nutrient composition of soybean cake: 44.1 CP, 9.2 NDF, 29.1 NFC, 10.2 EE, and 7.5 ash.

<sup>c</sup>NFC = non-fibrous carbohydrates = 100 - (CP + NDF + EE + ash).

## Feed Sampling and Calculation of DM Intake

Cows were fed once daily at 10:00–11:00 for *ad libitum* intake (5–10% orts). Diets were formulated using the Large Ruminant Nutrition System (LRNS; version 1.0.29) to meet the protein and energy requirements of a 550 kg cow producing 25 kg/d of milk (3.9% fat; 3.0% true protein) and consuming 18.5 kg DM/d, as according to the NRC (15). Due to protein content variation between the SBM and SBC, the four treatment diets were formulated to be iso-proteic, hence treatment diets differed in concentrate DM content (Table 1).

The freestall was fitted with 32 electronic feed bins and head gates (AF-1000-MG, Intergado Ltd., Contagem, Minas Gerais, Brazil), as well as six electronic water troughs. Feed and water troughs were attached to weight measurement platforms (WD-1000, Intergado Ltd., Contagem, Minas Gerais, Brazil) and radio frequency identification (RFID) antennas that monitored individual feed and water intake, as well as feeding and drinking behavior (16). Cows were fitted with an ear tag containing a unique passive transponder (FDX-ISO 11784/11785; Allflex, Joinville, SC, Brazil) in the right ear, and each feed bin was randomly assigned to a single cow. Each calorimetric chamber was also fitted with the Intergado feed technology and water trough. Chamber DMI was measured during the 2 days in which

the cows were in the chamber. Face-mask DMI was considered to be the intake of the cow in the free stall for the 2 days in which the animal underwent face-mask measurement.

Feed bin construction, dimensions and operation are as described by Chizzotti et al. (16). The visit duration, the number of visits to feed and water troughs, and fresh feed and water intake data were exported from Intergado web software for report generation. Body weight was also recorded each time cows consumed water via the platform with load cells associated with the water bins and exported from Intergado web software.

Dietary forage, concentrate mix, and orts were sampled weekly for DM determination. Diet intake for each cow was calculated using the Intergado system output and laboratory DM of diets.

## Collection of Rumen Samples

Rumen samples (60 mL/cow) were collected on the last week of the experimental trial, 4 h after feeding via a stomach tube (17, 18). Samples were preserved for volatile fatty acids (VFA) analysis by adding 1 mL of 20% (w/vol) metaphosphoric acid to 5 mL of sample (1:5 dilution). Samples were frozen at  $-20^{\circ}\text{C}$  until analysis.

## Collection of Blood Samples

During the first 7 days of the measurement period, blood samples were collected from the coccygeal vein, 2 and 6 h after feeding. Blood was collected in 4 mL vacutainer tubes (BD vacutainer systems, Plymouth, UK) and serum was extracted following centrifugation at  $1,800 \times g$  for 20 min at  $4^{\circ}\text{C}$ . Samples were stored at  $-20^{\circ}\text{C}$  until analysis.

## Milk Yield and Collection of Milk Samples

Cows were milked twice daily at 06:00 and 14:30 h. Milk yield was automatically recorded for each cow at each milking (DeLaval Alpro MM27BC milk meter system; DeLaval International, Tumba, Sweden). Composite milk samples (100 mL) were collected weekly at both a.m. and p.m. milkings for 3 consecutive days during each week of the measurement period, pooled and then analyzed for fat, protein, lactose, and urea-N content. These samples were preserved with Bronopol<sup>®</sup> (0.5 g/100 mL of milk) and stored at  $4^{\circ}\text{C}$  until analysis. An additional set of milk samples were collected separately at a.m. and p.m. milkings in 15-mL flasks containing no preservatives. These samples were immediately frozen at  $-20^{\circ}\text{C}$  until analyzed for fatty acid composition.

Milk composition was corrected for volume differences between the a.m. and p.m. milking. Total milk production was corrected by adjusting the fat content to 4% (FCM<sub>4%</sub>; fat-corrected milk) by the equation (15):

$$\text{FCM}_{4\%} = (0.4 \times \text{kg/d milk}) + \left[ 15 \times \frac{(\text{fat production} \times \text{milk yield})}{100} / 7 \right]$$

## Collection of Fecal and Urine Samples

Fecal samples were taken twice daily for 5 days at 09:00 and 14:00 directly from the rectum and frozen at  $-20^{\circ}\text{C}$ . Acid-insoluble ash was used as internal marker for total-tract digestibility

estimation. Fecal samples were analyzed for DM, OM, N, NDF, and ether extract content. Urine samples were collected once a day, across 3 consecutive days. A subsample of pooled urine was acidified with H<sub>2</sub>SO<sub>4</sub> to evaluate creatinine concentrations (19) and urine N content (AOAC, method 954.01). Daily urine volume (DUV, kg/d) was estimated by metabolic weight (BW<sup>0.75</sup>) and urinary creatinine concentrations according to the equation proposed by Valadares et al. (20):

$$\text{DUV} = \frac{\text{BW}^{0.75} \times 29}{\text{creatinine concentration (mg/L)}}$$

## CH<sub>4</sub> Measurement in Respiration Chambers

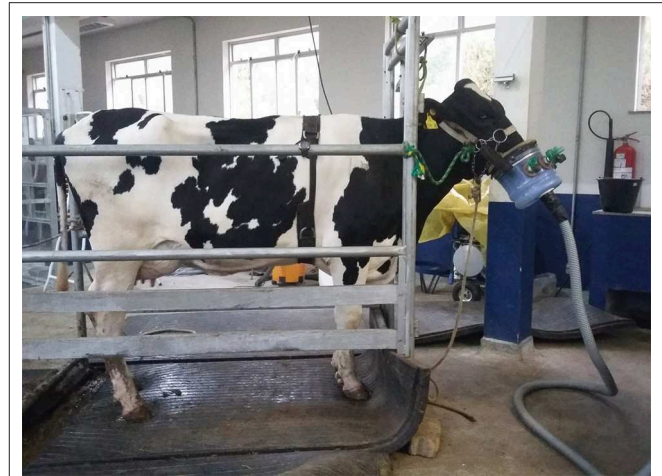
During the last weeks of measurements cows were randomly selected (one animal per treatment per chamber per collection period) and moved to an open circuit respiration chamber for 2 × 20 to 22 h periods for determination of CH<sub>4</sub> and CO<sub>2</sub> production, and O<sub>2</sub> consumption. All cows were trained and pre-conditioned for 2 weeks to the chamber and face-mask technique prior to the onset of the experiment. The four chambers were equipped as described by Machado et al. (21). Chambers were built in a pair wise manner with one cow housed per chamber. Days in which cows entered the chambers were staggered across 4 weeks, as there were only four chambers available at onetime. Each cow entered the chamber after the a.m. milking (06:00) and was removed for 2 h during the measurement period for p.m. milking (14:30). The chamber doors remained open during this time for cleaning and provision of fresh feed, twice a day from here on forward. Upon re-entry to the chamber, conditions were assumed to stabilize after 30 min. The chambers were maintained under thermal neutral conditions for crossbred Holstein × Gyr cows (Temperature: 23 ± 1°C; Relative humidity: 65 ± 5%). The animals were weighed before and after entering the chamber. The gas exchanges (O<sub>2</sub> input, CO<sub>2</sub> and CH<sub>4</sub> output) were calculated according to Machado et al. (21). Heat production (HP, Kcal/day) was calculated as according to Brouwer (22):

$$\text{HP(Kcal/d)} = (3.866 \times \text{VO}_2) + (1.200 \times \text{VCO}_2) - (0.518 \times \text{VCH}_4) - (1.431 \times \text{UN})$$

Where: VO<sub>2</sub> = volume of oxygen (L/d); VCO<sub>2</sub> = volume of carbon dioxide (L/d); VCH<sub>4</sub> = volume of methane (L/d); UN = total urine nitrogen (g/d).

## Methane Estimation Using a Face-Mask Technique

For spot measurements of CH<sub>4</sub>, CO<sub>2</sub>, and O<sub>2</sub> exchange, a face-mask method was employed. The mask was built using a 8 L polyethylene water container fitted with uni-directional valves (Figure 1) which prevented the rebreathing of exhaled air and allowed the external air to enter the mask (Era Mask, 220 mm × 160 mm × 77 mm, Biomedtech, Victoria, Australia). One cow at a time was taken to the controlled environment facility and placed within a squeeze chute for face-mask measurements. Through an inflatable circular rubber tube positioned around the animal's



**FIGURE 1** | Galvanized iron frame for animal restraint, and face-mask construction and application for the estimation of daily CH<sub>4</sub> methane production in cattle. Cow is also fitted with a heart rate monitor.

muzzle, the mask remained inflated and was positioned by a nylon strap attached around the neck of the cow (Figure 1). Gas sampling was performed by a tube that connected the mask to the flux meter and then gas analyzers, and sampling was performed at a rate of 0.3 L/min for each kg of live weight [Sable International Systems, Las Vegas, USA; (13)]. The calibration procedures of the system, sampling and data analysis were as described by Oss et al. (13). Briefly, the 30 min period consisted of a 5 min baseline, 20 min mask exhaust measurement, and another 5 min baseline. Measurement using the face-mask was conducted on 2 consecutive days, 4 h post feeding to avoid sampling at peak CH<sub>4</sub> production soon after feeding. This prevented the over estimation of daily CH<sub>4</sub> emissions of each cow. The CH<sub>4</sub> emissions data was recorded with the Sable System (Sable Systems International, Las Vegas, NV, USA) attached to the face-mask. Air flow rate (100 L/min) through the mask was controlled and measured by a mass flow controller (Flow Kit 500H; Sable Systems International, Las Vegas, NV, USA). Gas samples from the face-mask and ambient air were continuously sampled through Bev-A-Line tubes at 1 min intervals. The CH<sub>4</sub> and CO<sub>2</sub> analyzers were calibrated daily using N gas to zero the analyzers. Methane production (VCH<sub>4</sub>; mL/min) was calculated as according to Oss et al. (13);

$$\text{VCH}_4 = [\text{STD}_{fr} \times (\text{CH}_{4fm} - \text{CH}_{4b})]$$

Where STD = standard deviation of the flow rate; CH<sub>4fm</sub> = CH<sub>4</sub> measured from face-mask; CH<sub>4b</sub> = CH<sub>4</sub> measured from baseline).

Daily CH<sub>4</sub> was calculated by multiplying VCH<sub>4</sub> by 1.44 to convert to L/d and then converted to g/d (1 g CH<sub>4</sub> = 1.4 L CH<sub>4</sub>). Cows were individually restrained in a galvanized iron frame measuring 202 × 81 × 171 cm (length × width × height; Figure 1) for the duration of the measurement period.

## Chemical Analyses

The DM content of feed and fecal samples were determined by oven-drying at 55°C for 72 h. Dried samples were ground through a 1-mm screen and analyzed for neutral detergent fiber (NDF) as described by Van Soest et al. (23), modified for an Ankom 200/220 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY, USA). Amylase was used but sodium sulfite was not included in the NDF analyses and is expressed inclusive of residual ash. Ash content was determined after 2 h of oxidation at 600°C in a muffle furnace (24) (method 942.05). Nitrogen was quantified by Kjeldahl method (method 984.13). Ether extract (EE) was determined by extraction with ether as described for lipid extraction [method 920.39; (24)]. Non-fibrous carbohydrate (NFC) was calculated as by Mertens (25):

$$\text{NFC} = 100 - (\text{CP} + \text{NDF} + \text{EE} + \text{Ash})$$

The VFA concentrations were analyzed using high-performance liquid chromatography (Dionex Ultimate 3000 Dual Detector HPLC—Dionex Corporation, Sunnyvale, CA, USA [Phenomenex Rezex ROA ion exchange column, 300 × 7.8 mm]). Blood samples were analyzed for non-esterified fatty acids (NEFA; Randox, County Antrim, UK),  $\beta$ -Hydroxybutyric acid (BHBA; RANBUT assay, Randox, County Antrim, UK), cholesterol, triglycerides and urea-N (Randox, County Antrim, UK) using a microplate spectrophotometer (Eon, BioTek, Winooski, USA).

A Bentley Fourier-Transform Infrared Spectrometer System (Bentley FTS, Bentley Instruments, Chaska, MN) was used to determine milk fat, protein, and lactose. A commercial calorimetry kit (Sigma Diagnostics, St. Louis, MO) was used to analyze milk samples for urea-N. Milk FA composition was determined as described elsewhere (26). The Kjeldahl method (AOAC, method 954.01) was used to determine fecal N content and total fecal production was estimated by the insoluble ash technique (27).

## Statistical Analysis

The raw intake data obtained from the Intergado system were used to calculate total daily feed and water intake, the number of feed and water bin visits with and without consumption, and the duration of feed and water bin visits.

Data were analyzed with cow as the experiment unit for all variables. Feeding behavior, rumen fermentation parameters, and digestibility data were analyzed as a completely randomized design using the mixed procedure of SAS (28) with treatment as a fixed effect and cow nested within group as a random effect. For CH<sub>4</sub> measurement data, treatment, technique, and the interaction of treatment × technique were included as fixed terms and day of measurement was treated as a repeated measure. Time of sampling was treated as a repeated measure for blood variables. Since milk samples were collected separately at a.m. and p.m. milkings for fatty acid analysis, treatment, milking time and interaction were used as fixed term. Minimum values of Akaike's Information Criterion were used to choose covariance structure for each repeated measures analysis. Orthogonal polynomial contrasts were used to determine linear (L) and quadratic

(Q) responses of SBC (0, 6, 14, and 23% of SBC) replacing SBM. Significance was declared if  $P \leq 0.05$  and tendencies  $0.05 < P \leq 0.10$ .

The validity of the face-mask method to measure CH<sub>4</sub> was evaluated by regressing residuals (respiration chamber—face-mask) as a function of face-mask centered predicted values (29, 30) using the REG procedure (SAS, 9.4). Regressions with both intercept (mean bias) and slope (slope bias) not different from zero ( $P > 0.05$ ) were classified as potential accurate predicting equations.

## RESULTS

### Animal Performance

There was no effect ( $P \geq 0.21$ ) of replacing SBM with SBC on dry matter intake (DMI), feeding behavior, water consumption or BW (Table 2). Replacing SBM with SBC also had no effect on total VFA production ( $P = 0.79$ ) or the concentration of acetate, propionate and butyrate ( $P \geq 0.27$ ). There was a tendency ( $P = 0.08$ ) for lactate to decrease when 6% of SBC replaced SBM in the diet. Blood metabolites including BHBA, NEFA, Triglycerides, and cholesterol were not affected ( $P \geq 0.17$ ) by any treatment (Table 3). Serum concentrations of BHBA, NEFA, triglycerides, or cholesterol did not present an isolated treatment or collection time effect ( $P \geq 0.67$ ). There was interaction effect ( $P \leq 0.03$ ) of treatment × hour for NEFA and cholesterol. The 14% SBC and 23% SBC treatments resulted in a lower ( $P < 0.01$ ) serum cholesterol concentration at 6 h post-feeding compared to 2 h post-feeding, while NEFA concentration for cows fed the control diet was greater ( $P < 0.01$ ) at 6 h post-feeding only. The urea levels differed in relation to the time of collection ( $P < 0.01$ ) and presented a reduction trend ( $P = 0.07$ ) with the increase of SBC in the diet.

Replacing SBM with SBC had no effect on total daily FCM<sub>4%</sub> ( $P = 0.29$ ; Table 4). Milk protein, lactose, and urea concentrations were unaffected ( $P \geq 0.27$ ) by treatments; however, there was a trend ( $P = 0.09$ ) toward reduced total milk solids and fat percentage as SBC increased.

The replacement of SBM with SBC decreased ( $P \leq 0.05$ ) the contents of *iso* C14:0, *iso* C15:0, *iso* C16:0, *iso* C18:0, C16:0, *trans*-10, *cis*-12 CLA, C21:0, and total SFA in milk fat, but increased ( $P \leq 0.05$ ) the contents of C18:0, *cis*-9 C18:1, most *trans*-C18:1 isomers (except *trans*-4 and *trans*-5 C18:1), *cis*-11 C18:1, *cis*-12 C18:1, C18:2 n-6, *cis*-9, *trans*-11 CLA, total MUFA, total PUFA, and the n-6:n-3 FA ratio (Table 4). There was no effect of replacing SBM with SBC on apparent nutrient digestibility (Table 5).

### Methane Measurements and Techniques

The replacement of SBM by SBC had no effect ( $P > 0.23$ ) on DMI, FCM<sub>4%</sub> yield (kg/d), CH<sub>4</sub> production (g/d, g/kg BW<sup>0.75</sup>), CO<sub>2</sub> production, O<sub>2</sub> consumption or heat production. However, 14% SBC reduced ( $P \leq 0.01$ ) CH<sub>4</sub> yield (g/kg DMI) and both 14% SBC and 23% SBC reduced CH<sub>4</sub> intensity (g/kg milk; Table 6). The chamber technique for CH<sub>4</sub> measurement resulted in a decrease ( $P = 0.01$ ) in DMI (kg/d; %BW) and an increase in FCM<sub>4%</sub> ( $P = 0.05$ ). There was no technique effect ( $P \geq 0.24$ ) on CH<sub>4</sub>

**TABLE 2** | Effect of replacing soybean meal (SBM) with soybean cake (SBC) on dry matter intake (DMI), feeding behavior, water consumption, and body weight (BW) in dairy cattle.

	Treatments				SEM	P-value
	Control	6% SBC	14% SBC	23% SBC		
BW (kg)	614.4	578.4	577.3	613.5	17.95	0.28
<b>Feeding behavior</b>						
DMI (kg/d)	17.4	15.8	16.1	15.9	1.13	0.75
Total visits to feed bin	41.4	28.7	40.8	41.6	7.11	0.52
Number of feed bins visited	2.4	2.2	2.8	3.2	0.49	0.45
Visits with consumption	34.1	22.5	29.1	25.6	4.65	0.35
Visits without consumption	7.3	6.4	11.9	16.0	4.68	0.45
Total time spent at feed bin (h)	2.6	2.5	2.7	2.3	0.23	0.73
Total time spent eating (h)	2.6	2.5	2.6	2.3	0.23	0.70
<b>Drinking behavior</b>						
Water intake (L/d)	59.0	60.0	59.1	59.8	3.52	0.99
Total visits to trough	4.4	4.2	5.1	3.9	0.41	0.21
Visits with consumption	4.3	4.2	5.0	3.8	0.40	0.22
Visits without consumption	0.12	0.04	0.13	0.13	0.05	0.50
Total time spent at trough (h)	0.43	0.40	0.61	0.45	0.10	0.47
Total time spent drinking (h)	0.42	0.40	0.60	0.44	0.10	0.45

Control, no SBC; 6% SBC, 6% DM of soybean cake replacing soybean meal; 14% SBC, 14% DM of soybean cake replacing soybean meal; 23% SBC, 23% DM of soybean cake replacing soybean meal.

**TABLE 3** | Effect of replacing soybean meal (SBM) with soybean cake (SBC) on volatile fatty acids (VFA) and blood metabolites in dairy cattle.

Item	Treatment				SEM	P-value
	Control	6% SBC	14% SBC	23% SBC		
Total VFA (mM)	72.2	75.5	68.5	69.7	5.30	0.79
<b>VFA, mol/100 mol</b>						
Acetate (A)	63.2	61.7	62.6	63.1	0.68	0.37
Propionate (P)	22.7	23.3	22.0	21.9	0.62	0.36
Butyrate	14.0	15.1	15.4	15.0	0.52	0.27
Lactate	7.5	4.1	6.9	7.3	1.04	0.08
A:P ratio	2.8	2.7	2.9	2.9	0.09	0.39
<b>Blood metabolites</b>						
BHBA (mmol/L)	1.1	1.0	1.1	1.0	0.07	0.68
NEFA (mmol/L)	0.2	0.2	0.2	0.2	0.01	0.45
Triglycerides (mg/dL)	10.4	11.1	10.5	11.6	0.51	0.29
Cholesterol (mg/dL)	178.3	152.1	134.6	173.9	15.37	0.17
PUN (mg/dL)	51.0	46.3	41.8	43.7	2.59	0.07

Control, no SBC; 6% SBC, 6% DM of soybean cake replacing soybean meal; 14% SBC, 14% DM of soybean cake replacing soybean meal; 23% SBC, 23% DM of soybean cake replacing soybean meal.

BHBA,  $\beta$ -hydroxybutyrate; NEFA, non-esterified fatty acids; PUN, plasma urea nitrogen.

Treatment  $\times$  Time effect NEFA,  $P = 0.03$ ; Cholesterol Treatment  $\times$  Time effect,  $P = 0.001$ .

production reported as g/d or g/kg BW<sup>0.75</sup>, however there was an effect of technique ( $P = 0.01$ ) on CH<sub>4</sub> reported as g/kg DMI. The mask method underestimated ( $P < 0.01$ ) CH<sub>4</sub> (g/kg DMI) by 9.1% as compared to the chamber.

The production of CO<sub>2</sub> was not affected ( $P \geq 0.23$ ) by SBC inclusion, however there was a technique effect ( $P = 0.01$ ) when CO<sub>2</sub> was expressed per g/kg of DMI. The dietary treatments

had no effect on O<sub>2</sub> (g/d) consumption, however the mask overestimated ( $P = 0.04$ ) O<sub>2</sub> consumption (Table 6). Replacing SBM with SBC had no effect ( $P = 0.99$ ) on heat production with only a trend ( $P = 0.08$ ) for the technique to alter heat production.

Mean biases were significant for CH<sub>4</sub>, g/kg DMI and heat production, Kcal/BW<sup>0.75</sup> ( $P < 0.01$ ; Table 7). All measurements were significant ( $P < 0.05$ ) for linear bias with a maximum

**TABLE 4** | Effect of replacing soybean meal (SBM) with soybean cake (SBC) on milk production, milk composition, and milk fatty acids of dairy cows.

	Treatment				SEM	P-value
	Control	6% SBC	14% SBC	23% SBC		
Fat corrected milk yield (FCM <sub>4%</sub> , kg/d)	19.4	21.5	21.3	19.4	1.03	0.29
<b>Milk composition (%)</b>						
Total milk solids	12.4	12.6	12.2	11.6	0.27	0.07
Fat	4.6	4.8	4.4	4	0.23	0.09
Protein	3.4	3.3	3.3	3.3	0.07	0.77
Lactose	4.4	4.4	4.5	4.3	0.07	0.51
Urea	19.3	17.7	17	16.1	1.16	0.27
<b>Fatty acid (FA)</b>						
C4:0	3.56	3.55	3.54	3.46	0.119	0.91
C5:0	0.04 <sup>a</sup>	0.03 <sup>ab</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.004	0.01
C6:0	2.25	2.18	2.23	2.07	0.085	0.44
C7:0	0.04 <sup>a</sup>	0.03 <sup>ab</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.004	0.02
C8:0	1.35	1.28	1.36	1.19	0.067	0.23
C9:0	0.05	0.04	0.04	0.03	0.006	0.18
C10:0	2.96	2.67	3	2.58	0.199	0.32
C10:1 c-9	0.35	0.3	0.29	0.28	0.024	0.13
C11:0	0.08	0.07	0.08	0.08	0.008	0.55
C12:0	3.66	3.11	3.42	2.91	0.237	0.12
C12:1 c-9 + C13:0	0.25	0.2	0.21	0.19	0.021	0.21
C14:0 iso	0.11 <sup>a</sup>	0.10 <sup>ab</sup>	0.08 <sup>c</sup>	0.09 <sup>bc</sup>	0.007	0.01
C14:0	10.4	9.75	10.3	9.32	0.348	0.11
C15:0 iso	0.23 <sup>a</sup>	0.22 <sup>a</sup>	0.18 <sup>c</sup>	0.17 <sup>b</sup>	0.006	<.001
C15:0 anteiso	0.42	0.42	0.42	0.38	0.017	0.25
C14:1 c-9	1.33	1.05	0.97	1.15	0.103	0.07
C15:0	1.05	1	0.95	0.88	0.06	0.21
C16:0 iso	0.25 <sup>ab</sup>	0.25 <sup>a</sup>	0.20 <sup>c</sup>	0.21 <sup>bc</sup>	0.016	0.03
C16:0	29.6 <sup>a</sup>	27.6 <sup>ab</sup>	25.5 <sup>bc</sup>	24.9 <sup>c</sup>	0.819	<.001
C16:1 t-9 + C17:0 iso	0.33	0.35	0.29	0.31	0.016	0.05
C16:1 t-12	0.15	0.14	0.15	0.14	0.008	0.72
C16:1 c-9 + C17:0 anteiso	2.04 <sup>a</sup>	1.77 <sup>ab</sup>	1.53 <sup>b</sup>	1.67 <sup>b</sup>	0.125	0.03
C17:0	0.46 <sup>a</sup>	0.50 <sup>a</sup>	0.47 <sup>a</sup>	0.42 <sup>b</sup>	0.015	0.01
C18:0 iso	0.06 <sup>a</sup>	0.05 <sup>b</sup>	0.04 <sup>b</sup>	0.04 <sup>b</sup>	0.003	<0.01
C17:1 c-9	0.19	0.19	0.17	0.16	0.014	0.34
C18:0	9.0 <sup>b</sup>	11.4 <sup>a</sup>	11.5 <sup>a</sup>	11.4 <sup>a</sup>	0.58	0.01
C18:1 t-4	0.04	0.04	0.03	0.04	0.003	0.33
C18:1 t-5	0.03	0.03	0.03	0.02	0.003	0.19
C18:1 t-6, t-7, t-8	0.23 <sup>c</sup>	0.28 <sup>bc</sup>	0.33 <sup>ab</sup>	0.39 <sup>a</sup>	0.022	<0.01
C18:1 t-9	0.23 <sup>b</sup>	0.26 <sup>b</sup>	0.33 <sup>a</sup>	0.33 <sup>a</sup>	0.014	<.001
C18:1 t-10	0.28 <sup>c</sup>	0.38 <sup>bc</sup>	0.56 <sup>ab</sup>	0.76 <sup>a</sup>	0.092	<0.01
C18:1 t-11	0.86 <sup>b</sup>	1.05 <sup>b</sup>	1.43 <sup>a</sup>	1.48 <sup>a</sup>	0.111	<0.01
C18:1 t-12	0.29 <sup>d</sup>	0.38 <sup>c</sup>	0.49 <sup>b</sup>	0.55 <sup>a</sup>	0.023	<0.01
C18:1 t-13, t-14	0.40 <sup>b</sup>	0.42 <sup>b</sup>	0.48 <sup>ab</sup>	0.51 <sup>a</sup>	0.032	0.01
C18:1 c-9	19.0 <sup>b</sup>	20.9 <sup>ab</sup>	20.8 <sup>ab</sup>	22.8 <sup>a</sup>	0.789	0.01
C18:1 c-11	0.60 <sup>b</sup>	0.73 <sup>a</sup>	0.72 <sup>a</sup>	0.73 <sup>a</sup>	0.027	<0.01
C18:1 c-12	0.28 <sup>c</sup>	0.36 <sup>b</sup>	0.45 <sup>a</sup>	0.48 <sup>a</sup>	0.026	<0.01
C18:1 c-13	0.09	0.07	0.07	0.08	0.005	0.09
C18:1 t-16	0.24 <sup>d</sup>	0.27 <sup>c</sup>	0.32 <sup>b</sup>	0.36 <sup>a</sup>	0.011	<0.01
C19:0 + C18:1 c-15	0.08 <sup>a</sup>	0.07 <sup>b</sup>	0.08 <sup>ab</sup>	0.09 <sup>a</sup>	0.004	0.08

(Continued)

**TABLE 4 |** Continued

C18:2 <i>t</i> -9, <i>t</i> -12	0.03 <sup>a</sup>	0.02 <sup>b</sup>	0.02 <sup>c</sup>	0.02 <sup>bc</sup>	0.002	<0.01
C18:2 <i>c</i> -9, <i>t</i> -12	0.06 <sup>a</sup>	0.05 <sup>b</sup>	0.05 <sup>b</sup>	0.07 <sup>a</sup>	0.004	0.01
C18:2 <i>t</i> -9, <i>c</i> -12	0.05 <sup>a</sup>	0.03 <sup>b</sup>	0.02 <sup>c</sup>	0.02 <sup>bc</sup>	0.002	<0.01
C18:2 <i>n</i> -6	1.89 <sup>b</sup>	2.08 <sup>b</sup>	2.38 <sup>a</sup>	2.52 <sup>a</sup>	0.112	<0.01
C20:0	0.15	0.16	0.15	0.14	0.008	0.45
C18:3 <i>n</i> -6	0.05 <sup>a</sup>	0.04 <sup>ab</sup>	0.03 <sup>bc</sup>	0.03 <sup>c</sup>	0.003	<0.01
C18:3 <i>n</i> -3	0.31	0.35	0.36	0.37	0.016	0.06
C20:1 <i>c</i> -11	0.05	0.05	0.06	0.05	0.003	0.5
CLA <i>c</i> -9, <i>t</i> -11	0.49 <sup>b</sup>	0.54 <sup>b</sup>	0.72 <sup>a</sup>	0.84 <sup>a</sup>	0.048	<0.01
CLA <i>t</i> -9, <i>c</i> -11	0.04 <sup>a</sup>	0.03 <sup>b</sup>	0.03 <sup>b</sup>	0.03 <sup>ab</sup>	0.003	0.03
CLA <i>t</i> -10, <i>c</i> -12	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.01 <sup>b</sup>	0.01 <sup>b</sup>	0.003	<0.01
C21:0	0.04 <sup>a</sup>	0.03 <sup>b</sup>	0.02 <sup>bc</sup>	0.02 <sup>c</sup>	0.002	<0.01
C20:2 <i>n</i> -6	0.04 <sup>a</sup>	0.03 <sup>b</sup>	0.02 <sup>b</sup>	0.02 <sup>b</sup>	0.003	<0.01
C22:0	0.15 <sup>a</sup>	0.10 <sup>b</sup>	0.08 <sup>c</sup>	0.08 <sup>c</sup>	0.008	<0.01
C20:3 <i>n</i> -6	0.06	0.07	0.06	0.06	0.005	0.08
C20:4 <i>n</i> -6	0.18 <sup>a</sup>	0.13 <sup>b</sup>	0.1 <sup>c</sup>	0.09 <sup>c</sup>	0.011	<0.01
C23:0	0.05 <sup>a</sup>	0.04 <sup>b</sup>	0.03 <sup>c</sup>	0.03 <sup>c</sup>	0.004	<0.01
C20:5 <i>n</i> -3	0.06 <sup>a</sup>	0.04 <sup>b</sup>	0.03 <sup>c</sup>	0.03 <sup>c</sup>	0.003	<0.01
C24:0	0.05 <sup>a</sup>	0.05 <sup>a</sup>	0.03 <sup>a</sup>	0.03 <sup>a</sup>	0.005	<0.01
C22:5 <i>n</i> -3	0.06 <sup>ab</sup>	0.07 <sup>a</sup>	0.06 <sup>bc</sup>	0.05 <sup>c</sup>	0.003	0.01
Total SFA	66.1 <sup>a</sup>	64.6 <sup>a</sup>	63.7 <sup>a</sup>	60.4 <sup>b</sup>	1.058	<0.01
Total MUFA	24.6 <sup>c</sup>	26.9 <sup>bc</sup>	27.8 <sup>ab</sup>	30.4 <sup>a</sup>	0.921	<0.01
Total PUFA	3.36 <sup>c</sup>	3.53 <sup>bc</sup>	3.90 <sup>ab</sup>	4.12 <sup>a</sup>	0.175	0.01
Σ <i>n</i> -3FA	0.43	0.46	0.44	0.45	0.018	0.98
Σ <i>n</i> -6 FA	2.22 <sup>b</sup>	2.37 <sup>ab</sup>	2.61 <sup>a</sup>	2.70 <sup>a</sup>	0.122	0.03
<i>n</i> -6: <i>n</i> -3 FA ratio	5.24 <sup>b</sup>	5.17 <sup>b</sup>	6.0 <sup>a</sup>	6.08 <sup>a</sup>	0.195	<0.01

Control, no SBC; 6% SBC, 6% DM of soybean cake replacing soybean meal; 14% SBC, 14% DM of soybean cake replacing soybean meal; 23% SBC, 23% DM of soybean cake replacing soybean meal.

Means followed by a lowercase superscript are significant different at  $P < 0.05$ .

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

**TABLE 5 |** Effect of replacing soybean meal (SBM) with soybean cake (SBC) on apparently nutrient digestibility in dairy cows.

	Treatment				SEM	P-value
	Control	6% SBC	14% SBC	23% SBC		
<b>Apparent digestibility, %</b>						
Dry matter	81.9	81.7	81.6	81.9	0.70	0.98
Organic matter	82.6	82.6	82.4	82.6	0.73	0.99
Crude protein	83.9	83.0	81.9	82.7	0.72	0.24
Non-fiber carbohydrates	91.6	93.7	92.4	92.5	0.70	0.17
Neutral detergent fiber	67.9	65.2	66.9	66.9	1.24	0.50
Ether extract	85.8	85.7	85.3	86.9	1.52	0.89

Control, no SBC; 6% SBC, 6% DM of soybean cake replacing soybean meal; 14% SBC, 14% DM of soybean cake replacing soybean meal; 23% SBC, 23% DM of soybean cake replacing soybean meal.

bias of 102.8, 8.5, 5.5, 1.0, and 41.5 and a minimum bias of -75.5, -4.0, -6.9, -1.1, and -55.3 for CH<sub>4</sub> (g/d), CH<sub>4</sub> (g/kg DMI), CH<sub>4</sub> (g/kg milk), CH<sub>4</sub> (g/kg BW<sup>0.75</sup>), and heat production (Kcal/BW<sup>0.75</sup>), respectively (Table 7). The variation in CH<sub>4</sub> (g/d) observed in the chamber is shown in Figure 2. Methane measured in the chamber and by the face-mask at the same time (15:00 h) are the same. Plots of the regression of residuals on centered predicted values for CH<sub>4</sub> are shown

in Figure 3. Absence of bias occurred when intercept is equal to 0.

## DISCUSSION

The current study evaluated the potential of SBC to replace SBM in the diet of dairy cattle. Methane yield (g/kg DMI) was reduced by 11.6% when SBC replaced SBM at 14%, compared



**TABLE 6 |** Effect of replacing soybean meal (SBM) with soybean cake (SBC) on enteric CH<sub>4</sub> and CO<sub>2</sub> outputs, O<sub>2</sub> consumption and heat production in dairy cows.

	Treatment <sup>1</sup>				SEM	Technique			P-value	
	Control	6% SBC	14% SBC	23% SBC		Chamb	Mask	SEM	Treat	Tech
DMI (kg/d)	18.9	16.7	18.15	17.6	0.76	16.7 <sup>a</sup>	17.9 <sup>b</sup>	0.50	0.63	0.01
DMI (%BW)	2.95	3.02	3.35	3.01	0.16	3.0 <sup>a</sup>	3.2 <sup>b</sup>	0.10	0.30	0.01
FCM <sub>4%</sub> (kg/d)	17.9	19.0	21.1	19.9	1.30	20.2 <sup>a</sup>	18.8 <sup>b</sup>	0.78	0.37	0.05
<b>CH<sub>4</sub></b>										
g/day	354.2	354.0	333.8	334.5	13.54	342.6	345.7	9.96	0.54	0.80
g/kg BW <sup>0.75</sup>	3.0	3.11	2.95	2.81	0.104	2.97	2.98	0.077	0.24	0.92
g/kg DMI	20.7 <sup>ab</sup>	21.3 <sup>a</sup>	18.3 <sup>c</sup>	19.3 <sup>bc</sup>	0.67	20.9 <sup>a</sup>	19.0 <sup>b</sup>	0.50	0.01	<0.01
g/kg milk	19.6 <sup>a</sup>	18.7 <sup>ab</sup>	15.8 <sup>c</sup>	17.3 <sup>bc</sup>	0.81	17.4	18.3	0.60	0.01	0.25
<b>CO<sub>2</sub></b>										
g/day	11401	11289	11490	11314	406.0	11267	11482	278.0	0.98	0.49
g/kg BW <sup>0.75</sup>	96.9	99.2	101.7	95.1	3.18	97.5	99.0	2.20	0.49	0.55
g/kg DMI	663.8	680.2	632.8	657.4	18.25	681.9 <sup>a</sup>	635.2 <sup>b</sup>	13.62	0.33	0.01
g/kg milk	640.3	604.1	545.4	595.4	32.43	574.8	617.8	22.24	0.23	0.08
<b>O<sub>2</sub></b>										
g/day	7428	7362	7487	7408	21.5	7226 <sup>b</sup>	7616 <sup>a</sup>	168.5	0.99	0.04
g/kg BW <sup>0.75</sup>	63.1	64.7	66.3	62.3	1.93	62.6 <sup>b</sup>	65.7 <sup>a</sup>	1.33	0.46	0.04
g/kg DMI	431.4	444.7	413.3	429.8	12.39	437.8	421.8	9.24	0.37	0.17
g/kg milk	418.6	394.9	356	390.4	21.51	369.5 <sup>b</sup>	410.5 <sup>a</sup>	14.43	0.23	0.01
<b>Heat production</b>										
MJ/d	112.3	111.2	113.2	111.9	3.80	109.7	114.6	2.58	0.99	0.08
MJ/kg BW <sup>0.75</sup>	0.95	0.98	1.00	0.94	0.03	0.95	0.99	0.02	0.46	0.09

<sup>1</sup>Lsmeans of treatment effect (average values between chamber and face-mask techniques).

Control, no SBC; 6% SBC, 6% DM of soybean cake replacing soybean meal; 14% SBC, 14% DM of soybean cake replacing soybean meal; 23% SBC, 23% DM of soybean cake replacing soybean meal.

Chamb, Chamber technique; Mask, Face-mask technique; DMI, dry matter intake; BW<sup>0.75</sup>, metabolic body weight.

Means followed by a lowercase superscript are significantly different at P < 0.05.

FCM<sub>4%</sub>, fat correct milk yield.

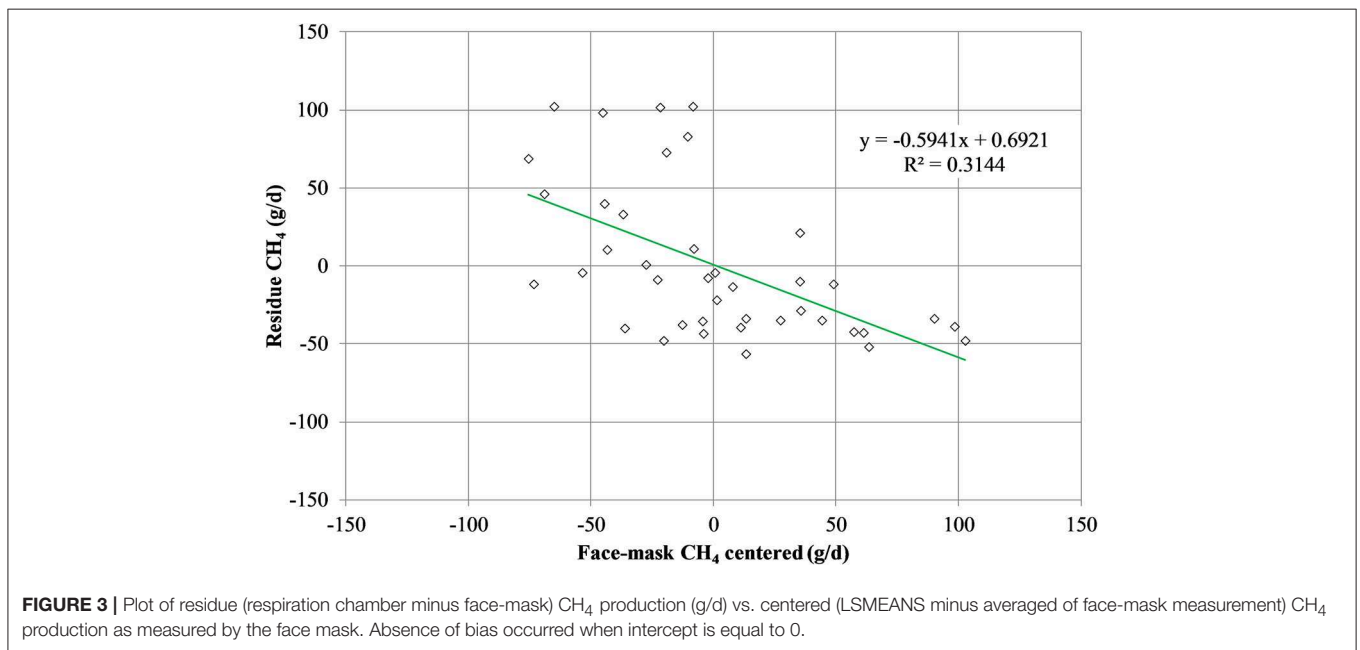
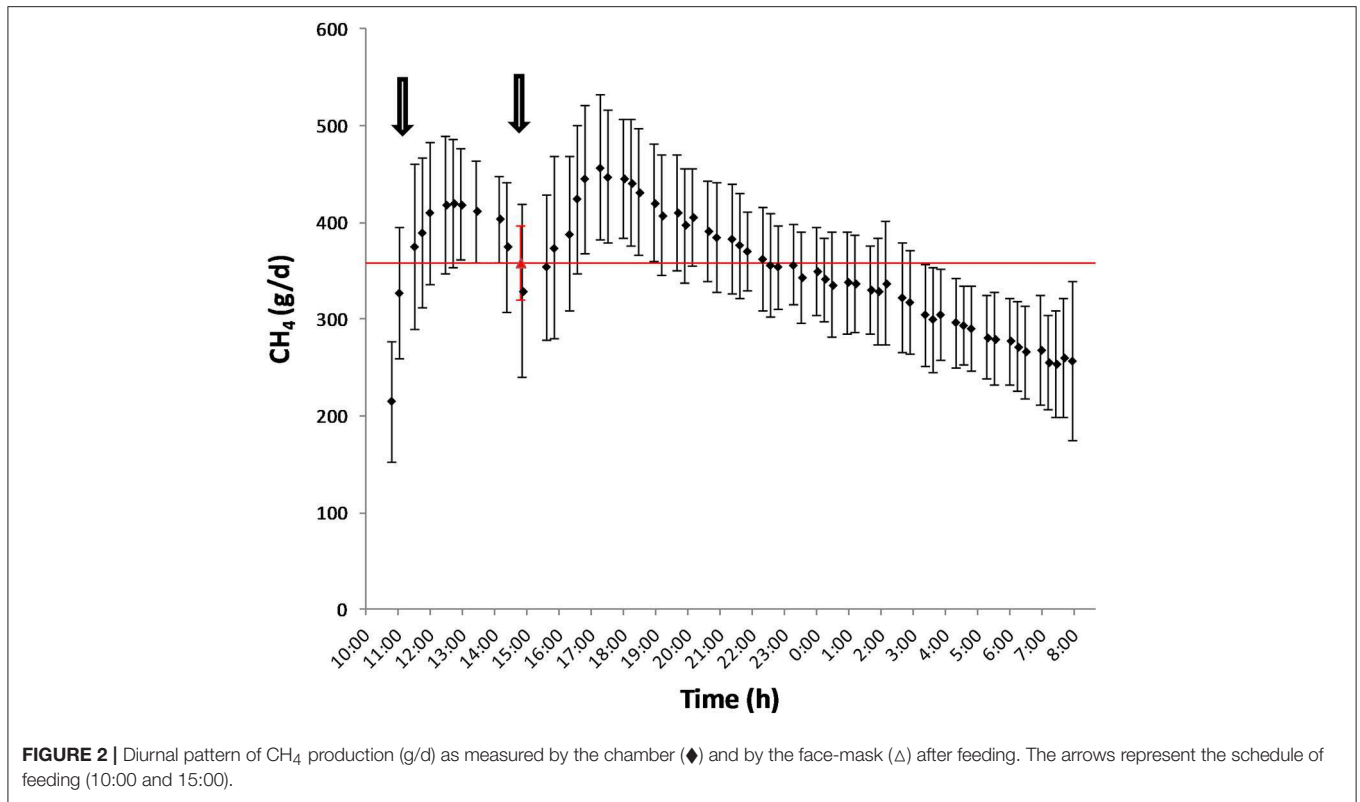
**TABLE 7 |** Evaluation of bias for CH<sub>4</sub> outputs (g/d, g/kg DMI, g/kg milk, and g/kg BW<sup>0.75</sup>) and heat production (Kcal/BW<sup>0.75</sup>) measured using face-mask and respiration chambers in dairy cows fed replacing concentrations of soybean meal (SBM) with soybean cake (SBC).

	Average CH <sub>4</sub>	Mean bias	P-value mean bias	Linear bias	P-value linear bias	Maximum bias	Minimum bias
CH <sub>4</sub> , g/d	358.5	1.17	0.850	-0.53	<0.001	102.8	-75.5
CH <sub>4</sub> , g/kg DMI	20.86	0.99	0.005	-0.97	<0.001	8.5	-4.0
CH <sub>4</sub> , g/kg milk	17.6	-0.70	0.073	-0.26	0.048	5.5	-6.9
CH <sub>4</sub> , g/kg BW <sup>0.75</sup>	3.08	0.01	0.918	-0.72	<0.001	1.0	-1.1
Heat production, Kcal/BW <sup>0.75</sup>	231.4	-9.41	<0.001	-0.66	<0.001	41.5	-55.3

DMI, dry matter intake; BW<sup>0.75</sup>, metabolic weight.

to the control. The decrease in CH<sub>4</sub> intensity (g/kg milk) was likely due to observed numerical increase in FCM<sub>4%</sub> and decrease in CH<sub>4</sub> (g/d). The 11.6% reduction in CH<sub>4</sub> yield as observed in this study is comparable to results reported by Beauchemin et al. (6), who found that CH<sub>4</sub> production was decreased by 16% in dairy cows when the diet was supplemented with canola meal as to increase the EE content of the diet to 3.9% (total diet DM). Brask et al. (31) examined different physical forms of rapeseed fat and found that CH<sub>4</sub> intensity (L/kg total ingested OM) was 12.6% lower for rapeseed cake than rapeseed meal. Martin et al. (32) showed that including crude linseed

reduced CH<sub>4</sub> emissions (g/kg DMI) by 10% with a total diet fat content of 5.7% in dairy cattle. Further reductions were observed when extruded flaxseeds (26%) or linseed oil (49%) replaced crude linseed, keeping the same total diet fat content. McGinn et al. (33) found that 5% sunflower oil inclusion in a forage based diet resulted in a 17% reduction in CH<sub>4</sub> (g/kg DMI) emissions. In the present study, 14% SBC had a larger reduction in CH<sub>4</sub> yield than 23% SBC. This was possibly due to the numerical decrease in DMI and FCM<sub>4%</sub> associated with the higher replacement concentration. The replacement of 6% SBM with SBC did not change CH<sub>4</sub> emissions compared to the



control, suggesting that the level of dietary fat was insufficient to inhibit methanogenesis.

The dietary treatments in this experiment were formulated to offer increasing dietary fat concentrations, so that the highest inclusion of SBC was lower than that as recommended by the National Research Council (15) of 6–7% total diet DM. This recommendation is given so that DMI or organic matter

fermentation is not negatively affected by dietary fat content (6, 7, 32). As such, there was no effect of SBC inclusion on DMI in the current study. Similarly, replacement of rapeseed meal with rapeseed cake did not alter OM or NDF digestibility in lactating Holstein cows when dietary fat content was below 6.5% of total diet DM (31). This is in contrast to the findings of Jordan et al. (34) who observed a reduction in DMI in bulls fed a high forage

diet that were supplemented with whole soybean. However, in that study, ether extract content was high (11.0%) which likely caused an inhibition of fiber digestion, increasing retention time, and contributing to rumen fill and decreased intake. Similarly, replacement of SBM with SBC in the present study did not alter nutrient digestibility or rumen fermentation.

The replacement of SBC with SBM decreased to varying degrees the contents of most iso fatty acids, C16:0, trans-10, cis-12 CLA, C21:0, and total SFA in milk fat, whereas an opposite effect was observed on milk fat contents of C18:0, cis-9 C18:1, most trans-C18:1 and cis-C18:1 isomers, C18:2 n-6, cis-9, trans-11 CLA, total MUFA, total PUFA, and the n-6:n-3 FA ratio. Except for the reduction in trans-10, cis-12 CLA, these effects are consistent with results from previous studies where plant oils rich in linoleic acid were added to dairy cow diets (35). The pronounced increase in milk fat C18:2 n-6 observed in cows fed the highest level of SBC (1.89 vs. 2.52 g/100 g of total FA for control and 23% SBC, respectively) indicates that part of the oil present in this feed ingredient escaped from rumen biohydrogenation, which is in accordance with several reports showing that milk fat C18:2 n-6 and C18:3 n-3 contents are increased to a larger extent when cows are fed with diets supplemented with whole or processed oilseeds when compared to plant oils (36). The gradual decrease in milk fat contents of iso C14:0 and iso C15:0 in response to dietary SBC inclusion is consistent with the concomitant reduction in methane output (expressed as g/kg of DM or g/kg of milk) observed in the present study; concentrations of these branched-chain fatty acids in milk fat were shown to be positively correlated to CH<sub>4</sub> emissions in previous studies (37).

The respiration chamber is currently established as the “gold standard” for quantifying CH<sub>4</sub> emissions from livestock (38), however there are disadvantages with its use. In this experiment it was found that DMI intake was 6.7% higher ( $P = 0.01$ ) in the cows undergoing the mask technology than that of the chamber. One of the main disadvantages of using the chamber technique to quantify CH<sub>4</sub> emissions is observed changes in behavior (12). Marked decreases in feed, as observed in the present study, and water intake can be observed with animals in respiration chambers, affecting CH<sub>4</sub> measurements due to the direct relationship between CH<sub>4</sub> emissions and these variables. Despite the difference in DMI between techniques, CH<sub>4</sub> production (g/d and g/kg BW<sup>0.75</sup>) was not different ( $P \geq 0.80$ ) between the techniques. However, the face-mask technique predicted 9.1% less methane than the chamber when quantified on a g/kg of DMI basis and a g/kg of milk basis, also predicting less O<sub>2</sub> consumption and CO<sub>2</sub> production. In comparison, Oss et al. (13) found that using the face-mask technique resulted in 4% lower CH<sub>4</sub> production (g/d) measured than that of the respiration chamber. The difference observed between these techniques may, in part, be due to flatus emissions which are not accounted for using this technique. However, this contribution is evaluated as being <2% of total CH<sub>4</sub> production emitted from the cow (39).

The main explanation for the difference in accuracy of CH<sub>4</sub> measurements obtained by the face-mask observed between Oss et al. (13) and the present study is the time at which face-mask

measurements were taken. Due to labor restrictions, face-mask measurements were conducted 4 h post feeding compared to 6 h after feeding as done by Oss et al. (13). The time after feeding has been proposed to be highly correlated with average daily CH<sub>4</sub> emissions (40, 41) and can account for the observed differences between these studies. Emission rates are known to follow momentary and diurnal patterns such that using a spot measurement as an average for daily production is not adequate for predicting CH<sub>4</sub> production (42). As seen in **Figure 2**, the rate of average CH<sub>4</sub> production for these animals followed the diurnal pattern in which CH<sub>4</sub> production increased after feeding (3 h), reached peak at 8 h post-feeding and then steadily decreased there on after. As a 20 min spot sampling conducted in the present study, and by Oss et al. (13), does not cover the duration of a feeding and activity cycle, prediction equations need to be developed (42) which account for these patterns.

The proposed face-mask method is less expensive and can be conducted over a shorter time. However, the accuracy and precision of spot sampling techniques to measure CH<sub>4</sub> emissions is still uncertain (42). Information provided by simple regression analysis can be ambiguous and lack sensitivity and, often, do not provide a proper interpretation of these relationships. The linear bias observed by the face-mask technique, though not observed by mixed model, reiterates the necessity of complex adjustment factor for daily emission calculations. However, the face-mask has shown to accurately measure emissions when conducted at the same time point as within the “gold standard” chamber. Inclusion of the uni-directional valves as an additional adjustment of the mask from Oss et al. (13), has improved the accuracy of measurement. The face-mask method in place of chamber measurements may alleviate some animal welfare concerns as animals are only strictly confined for 30 min, allowing normal behavior and activity for most of the day. However, further assessment on stress response to the face-mask technique is required.

Oss et al. (13) suggested that the face-mask technique presents a greater day and animal variation when compared to the SF<sub>6</sub> and respiration chamber techniques. This method of measurement is highly dependent on the timing of measurements due to the diurnal patterns of the feeding cycle and CH<sub>4</sub> emissions. However, these limitations may be overcome by obtaining data from a larger sample size of animals with strict timing of measurements as done in this experiment (43). The bias of the face-mask compared to the chamber could be minimized by increasing the number of animals used per treatment, as well as conducting two measurement periods per day (42). However, as previously discussed, increasing the times of measurements per day can also increase the risk of behavioral changes, decreasing the ability to accurately predict CH<sub>4</sub> emissions. Due to restrictions in labor and technical staff the current study did not conduct more sampling events over the day. Statistical methods to assess the validity of the face-mask to accurately predict daily CH<sub>4</sub> production can also alter the results as observed in this study. A standardized protocol for measurement and calculation of CH<sub>4</sub> production will allow future implementation of the face-mask methods for determining CH<sub>4</sub> production from ruminants.

## CONCLUSION

The replacement of SBM with 14% SBC reduced CH<sub>4</sub> yield (g/kg DMI) and intensity (g/kg milk) in dairy cows, without having a negative impact on animal intake, rumen metabolism, FCM<sub>4%</sub>, or nutrient digestibility. This presents SBC as a feasible alternative to SBM, with the additional benefit of decreasing enteric CH<sub>4</sub> production.

In the current study, the face-mask method was able to accurately predict daily CH<sub>4</sub> emissions from spot sampling. However, this is confounded by the linear bias when evaluated using regression analysis. Therefore, for the face-mask to be accepted as a standard CH<sub>4</sub> measurement tool, prediction equations need to be formulated which account for feeding behavior and the diurnal patterns of CH<sub>4</sub> production.

## DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

## ETHICS STATEMENT

All animal care and handling procedures were approved by the Embrapa Dairy Cattle Animal Care and Use Committee (Juiz de Fora, Minas Gerais, Brazil; Protocol No. 28/2014).

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## AUTHOR CONTRIBUTIONS

LGRP, AVC, RMM, TRT, FSM, and MMC: study design. SRS, RSR, JPS, ALF, RMM, and TRT: acquisition of data. SRS, RSR, ALF, and MASG: lab analysis. AVC: statistical analysis. SAT, TEB, AVC, LGRP, RMM, SRS, TRT, and MASG: writing the manuscript. All authors read, critically revised for intellectual contents and approved the final manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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