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Original article

Effect of feeding spent coffee grounds on the methane production in bovine rumen

K. Yamada[1], K. Kawai[2], Y. Inui[1], K. Oda[1], T. Kurumisawa[2], Y. Shimizu[2], Y. Shinozuka[2]

[1] Tsukuba Research Laboratories,
Tokuyama Corporation, 40 Wadai, Tsukuba, Ibaraki, 300-4247, Japan
[2] School of Veterinary Medicine,
Azabu University, 1-17-1 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa, 252-5201, Japan

Correspondence to: K. Kawai, e-mail: kawai@azabu-u.ac.jp

Abstract

The environmental impact of methane, a greenhouse gas emitted from ruminants, is a pressing issue and methods to control methane emissions from ruminants are being investigated worldwide. In this study, we investigated the effects of the administration of spent coffee grounds (SCG) on methane production in the rumen in two cows. In the control condition (days 1 and 2), the cows were fed a basic diet twice daily (roughage and concentrate), and in the SCG condition (days 1 and 2) sequentially, the cows were fed the same basic diet and administered SCG into the rumen twice daily. The methane and carbon dioxide concentrations in rumen gas were measured via a fistula after feeding on days 2 in both cases of the study. The measurements were made using a newly developed gas measurement system with a portable gas monitor, and data were obtained for the control condition and SCG condition at each measurement time. The methane ratio at each measurement time was calculated from the methane and carbon dioxide concentrations, and compared between the two conditions. Statistical analysis showed no significant difference between the two conditions in the methane ratios after the morning (P=0.108) and afternoon feedings (P=0.345). However, the methane ratios before the morning (P=0.043) and afternoon feedings (P=0.008) were significantly lower in the SCG condition than in the control condition, suggesting that the administration of SCG may suppress methane production in the rumen.

Keywords: bovine rumen, coffee grounds, greenhouse gases, methane measurement

Introduction

Anthropogenic greenhouse gas emissions are increasing year by year, contributing to the rise in global average temperatures. Methane, a greenhouse gas, has approximately 25 times the greenhouse effect of carbon dioxide (Rodhe 1990), and is considered to be as significant as carbon dioxide. According to the 2019 Greenhouse Gas Inventory Data of the United Nations Framework Convention on Climate Change, approximately 11% (carbon dioxide equivalent) of the greenhouse gas emissions in Annex 1 countries consists of methane, with the largest share (40.1%) coming from agriculture. Furthermore, approximately 78% of the agricultural emissions derive from fermentation in the digestive tract of livestock (United Nations Framework Convention on Climate Change [UNFCCC] 2019), making the reduction of methane emissions from livestock ruminants an important issue. Methane is produced by methanogenic archaea that use the hydrogen produced during feed digestion in the stomachs of livestock ruminants (Knapp et al. 2014). Therefore, it is considered that methane emissions can be reduced by decreasing the amount of hydrogen in the rumen. The antibiotic monensin, which has been used as a feed additive, has attracted attention for its methane-reducing effects (Russell and Strobel 1989). However, the European Union has banned the use of antibiotics, including monensin, as feed additives since 2006 due to the problem of antibiotic-resistant bacteria (European Commission 2015), and alternative materials are now being developed.

Currently, with the global expansion of coffee consumption, the processing of spent coffee grounds (SCG) as food residues has become a challenge. It has been reported that the feeding of silaged coffee grounds to dairy cows promotes antioxidant activity in blood and milk, and decreases the number of somatic cells in the milk of cows (Kawai et al. 2018); as such, the effective use of SCG as food residues is expected. Coffee beans are rich in chlorogenic acid, an antioxidant, which remains in the residue after coffee extraction (Murthy and Madhava 2012). The roasting of coffee beans may oxidize chlorogenic acid and produce chlorogenic acid quinone. Coffee beans also contain high levels of unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid. We hypothesized that the hydrogen produced in the rumen by these quinones and unsaturated fatty acids would reduce the partial pressure of hydrogen, and inhibit methanogenesis by methanogenic archaea. Thus, in this study, we examined the effects of SCG on reducing methane gas in rumen gas by administering SCG into the rumen of cows.

Various methods have been developed to measure the methane emissions from cattle, and many of them have been summarized, along with their advantages and disadvantages, by Johnson and Johnson (1995), Kebreab et al. (2006), Bhatta et al. (2007), and Storm et al. (2012). The use of a respiration calorimeter is a common method for measuring methane; however, although it can adequately measure methane production from the rumen and gut, it is expensive to construct and maintain a large chamber. The ventilated hood method and face mask method, which are less expensive to maintain than a chamber, estimate the rumen methane concentrations from the ratio of methane to carbon dioxide in the breath collected from a semi-enclosed space on the head or from a mask worn by the cow (Bhatta et al. 2007), but they do not directly measure rumen gas. The tracer method using sulfur hexafluoride as a reference material can be used to measure the methane emissions of free-ranging cattle in pastures, but the obtained results vary more than those obtained from chamber measurements (Storm et al. 2012), and sulfur hexafluoride may remain in the milk or meat of livestock (Bhatta et al. 2007). Therefore, as a more efficient method of measuring the methane emissions from cattle, we employed a new and simple measurement system that uses a commercially available portable gas monitor to directly measure the rumen gas composition.

Materials and Methods

Animals and materials

The Animal Experiment Committee of Azabu University provided ethical approval for this study (No. 200513-6 dated 20-05-2020).

The study was conducted in February 2021, and from July to August 2022, in a tie-stall barn at the Azabu University. Two Holstein dry cows (17 years old (700 kg body weight) and 3 years old (759 kg body weight)) with fistulas in the first stomach were used as test animals.

For the control condition, a basic diet consisting of a mixture of Sudan hay (3.5 kg) and concentrate (0.5 kg) was fed twice a day, at 8:00 and 15:30, to the cows. For the SCG condition, SCG (0.5 kg dry matter) were administered twice a day directly into the rumen through the fistula immediately after feeding of the basic diet. Cows were allowed to drink water freely. The nutrient compositions of the diets were analyzed by the Japan Food Research Laboratories [JFRL] (Tokyo, Japan) according to the Japan Analytical Standards of Feeds (Food and Agricultural Materials Inspection Center [FAMIC] 2020). Dry matter of each feed was calculated by subtracting moisture content. Neutral

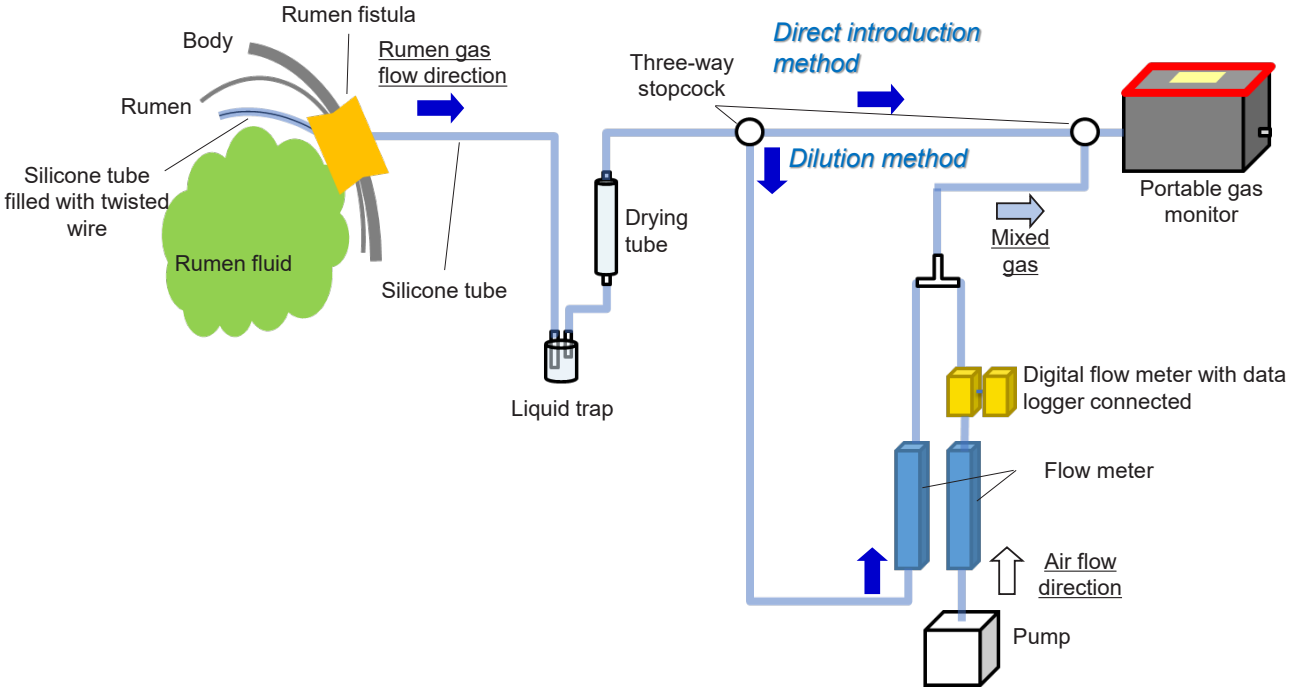

Fig. 1. Schematic diagram of the rumen gas measuring device.
Since the carbon dioxide detection range of the gas monitor was 0 to 20 vol%, a rumen gas dilution mechanism using air was installed to allow simultaneous detection of methane and carbon dioxide when the carbon dioxide concentration in the rumen gas exceeded 20 vol%.

detergent fiber and acid detergent fiber were analyzed by the method of Van Soest and McQueen. (1973).

Dried spent coffee grounds

The dried SCG were prepared by extracting regular coffee in hot water at 100°C for 5 min, filtering, and drying the residue in an air dryer at 40°C until the moisture content reached 9% to 10%. The amount of chlorogenic acid in the prepared SCG were analyzed by JFRL using high-performance liquid chromatography-mass spectrometry. Analytical specifications: - Column: Mightysil RP-18 GP (5 µm, i.d. 2.0 mm × 150 mm, Kanto chemical co., inc., Tokyo, Japan); mobile phase- water: acetonitrile: acetic acid (1000:50:3) [v/v]; eluent flow rate: 0.2 mL/min; column temperature: 30°C; ion source: electrospray ionization; mass analyzer: tandem mass spectrometry. Fatty acid composition was determined by JFRL using acid hydrolysis method in accordance with the Japanese food labeling standards.

Measurements

A schematic diagram of the rumen gas-measuring device is shown in Fig. 1. A portable gas monitor with an internal pump (RX-8500, Riken Keiki, Tokyo, Japan) was used to measure the amounts of methane and carbon dioxide in the rumen gas aspirated from the fistula. The gas monitors had a methane detection range of 0 to 100 vol%, and a carbon dioxide detection range of 0 to 20 vol%; both were determined by a non-dispersive infrared method. The gas aspiration rate was 750 mL/min. A silicone tube with an inner diameter of 5 mm was used to supply rumen gas to the gas monitor. To prevent the tip of the silicone tubing from being buried in the rumen contents, a twisted stainless steel wire was inserted into the tubing to maintain its shape. The silicone tubing was inserted into the rumen through a fistula lid with a hole that was 10 mm in diameter. Plumbing putty was applied to the gap between the lid and the tube to prevent gas leakage. In the direct method, the sample gas was introduced into the gas monitor through the silicone tube inserted in the rumen, a liquid trap, and a dry tube filled with silica gel. The liquid trap and the drying tube were introduced to prevent water vapors from affecting the detection intensity, since the gas monitor uses an infrared measurement system. In addition, since the carbon dioxide upper detection limit of the gas monitor was 20 vol%, a rumen gas dilution mechanism was installed to allow simultaneous detection of methane and carbon dioxide when the carbon dioxide concentration in the rumen exceeded 20 vol%. Specifically, three-way stopcocks were installed in the flow path of the gas monitor. Rumen gas was introduced through a flow meter for flow confirmation on one side, and air from a pump was introduced on the other side, with the flow rate controlled by a flow meter to dilute the rumen gas by approximately 6 times. The flow rate of air through the

Table 1. Schedule of feeding diet (basic diet & SCG) and analysis (rumen gas measurement).

Condition	Day / Time	7:30	8:00	11:00	15:00	15:30	18:30
Basic diet (control)	Day-1	-	Feeding	-	-	Feeding	-
	Day-2	Analysis	Feeding	Analysis	Analysis	-	Analysis
Basic diet with spent coffee grounds (SCG)	Day-1	-	Feeding	-	-	Feeding	-
	Day-2	Analysis	Feeding	Analysis	Analysis	-	Analysis

Basic diet (control condition) and SCG administered diet was fed on same time on both days. Analysis was also performed on both days on same time to see the real impact of SCG administered diet.

flow meter was recorded by a digital flow meter (PF2M701, SMC, Tokyo, Japan) and logger (LR5031, HIOKI E.E., Nagano, Japan). The gas monitor was calibrated by exposing it to a known concentration of calibration gases (methane: 3.54 vol% and 70.1 vol%; carbon dioxide: 10.1 vol%, Riken Keiki, Tokyo, Japan) for a specific amount of time. The dilution system was validated by measuring methane calibration gas (3.54 vol% or 70.1 vol%) and air mixed in certain proportions.

The processed fistula lid was attached only at the time of measurement. The amounts of methane and carbon dioxide in the rumen gas were measured by the dilution method and the direct method for a total of approximately 20 min until the effect of the lid replacement was no longer observed. The methane ratio was calculated from the methane and carbon dioxide concentrations measured by the dilution method or the direct method with Equation (1), and the mean value of the methane ratio was calculated for a time period of approximately 1 to 3 min when the rate of change in values per 10 s was less than 1%. If feed remained in the feed bunk or the methane ratio did not reach equilibrium, the corresponding 1-day data were excluded.

The methane reduction rate was calculated from the difference in the methane ratio between the control condition and the SCG condition based on Equation (2).

Methane ratio (%) = Methane concentration (vol%) / Carbon dioxide concentration (vol%) × 100 (Equation 1)

Methane reduction rate = (Methane ratio in the control condition - Methane ratio in the SCG condition) / Methane ratio in the control condition × 100 (Equation 2)

Experimental design

Previous studies that measured gas production in lactating cows using the respiration chamber and head box methods have shown that there is diurnal variation in the gas production in the rumen (Kume et al. 2003, Bell et al. 2018). Therefore, it was expected that gas production would increase as digestion of the feed progressed, reaching a maximum at 2 to 3 h after feeding, and be the lowest before feeding. Therefore, in this experiment, measurements were conducted at four time points: 3 h after feeding, which was considered to be the time at which the amount of gas generation would be the highest, and 30 min before feeding, which was considered to be the time at which the amount of gas generation would be the lowest, for both the morning and afternoon feedings. Furthermore, since the interval between the morning and afternoon feeding times was 7.5 h, and the interval between the afternoon and morning feeding times was 16.5 h, the data were analyzed separately for the morning and afternoon periods.

The experiments shown in Table 1 were performed using the two cows. For the control condition, which was examined on days 1 and 2, the basic diet was fed twice a day, once in the morning and once in the afternoon. For the SCG condition, which was examined on days 1 and 2, the cows were fed the basic diet and dried SCG was administered into the rumen twice a day, once in the morning and once in the afternoon. The rumen gas composition was measured 30 min before the morning feedings on days 2 in both cases of the study for approximately 20 min. During the measurements, the cows were prevented from drinking water. After the measurements were finished, the cows were fed the basic diet or fed the basic diet and administered dried SCG in the same manner as on days 1 in both cases, respectively. Three hours after the feeding time, the rumen gas composition was measured for approximately 20 min. The afternoon feedings and measurements were conducted in the same manner as in the morning.

Statistical analysis

Statistical analysis was performed using the Mann-Whitney U test. p-values <0.05 were considered to indicate statistical significance (SPSS 22 software, IBMCorp., Armonk, NY, USA).

Results

Composition analysis of the basic diets and SCG

The nutrient composition of the diets is shown in Table 2. The results of the SCG analysis are shown in Tables 3 and 4. The chlorogenic acid content per 100 g of coffee grounds was 132 mg/100 g. Among the

Table 2. Nutrient composition of diet feed.

	Sudan grass	Concentarte	Spent coffee grounds (SCG)
Dry Matter (%)	90.3	86.7	90.5
Crude Protein (% on DM)	3.3	18.1	13.7
Crude Fat (% on DM)	1.1	2.9	11.4
Crude Ash (% on DM)	6.6	5.1	1.3
NDF (% on DM)	64.6	15.9	64.1
ADF (% on DM)	39.3	8.7	42.7

NDF – neutral detergent fiber, ADF – acid detergent fiber.
Details of nutrient composition of diet feed are mentioned.

Table 3. Amount of chlorogenic acids in spent coffee grounds (SCG).

Chlorogenic acids	mg/100 g
3-*O*-caffeoylquinic acid (3-CQA)	26
4-*O*-caffeoylquinic acid (4-CQA)	33
5-*O*-caffeoylquinic acid (5-CQA)	45
3-*O*-feruloylquinic acid (3-FQA)	7
4-*O*-feruloylquinic acid (4-FQA)	8
5-*O*-feruloylquinic acid (5-FQA)	13

Varying forms of chlorogenic acids content in SCG diet are enclosed.

Table 4. Fatty acid composition of spent coffee grounds (SCG).

Fatty acids (Common name)	Lipid Numbers	Total fatty acid (%)
Myristic acid	14:0	0.1
Palmitic acid	16:0	34
Margaric acid	17:0	0.1
Stearic acid	18:0	7.1
Oleic acid	18:1	10.1
Linoleic acid	18:2n-6	43.1
Linolenic acid	18:3n-3	1.2
Arachidic acid	20:0	2.9
Eicosenoic acid	20:1	0.3
Behenic acid	22:0	0.6
Lignoceric acid	24:0	0.3
Unidentified		0.1

Summary of fatty acid composition has been stated.

fatty acids, the unsaturated fatty acids included oleic acid (18:1; 10.1%), linoleic acid (18:2n-6; 43.1%), and linolenic acid (18:3n-3; 1.2%).

Determination of the gas concentration in the rumen with the new measurement system

Fig. 2 shows an example of the methane and carbon dioxide measurements taken first by the dilution method, then by the direct method, as well as the calculated methane ratios. The concentrations of methane and carbon dioxide measured by the dilution method continued to increase slowly after the start of the measurements. After switching to the direct method, the methane concentration continued to increase gradually until the end of the measurement period. On the other hand, the carbon dioxide concentration exceeded the upper detection

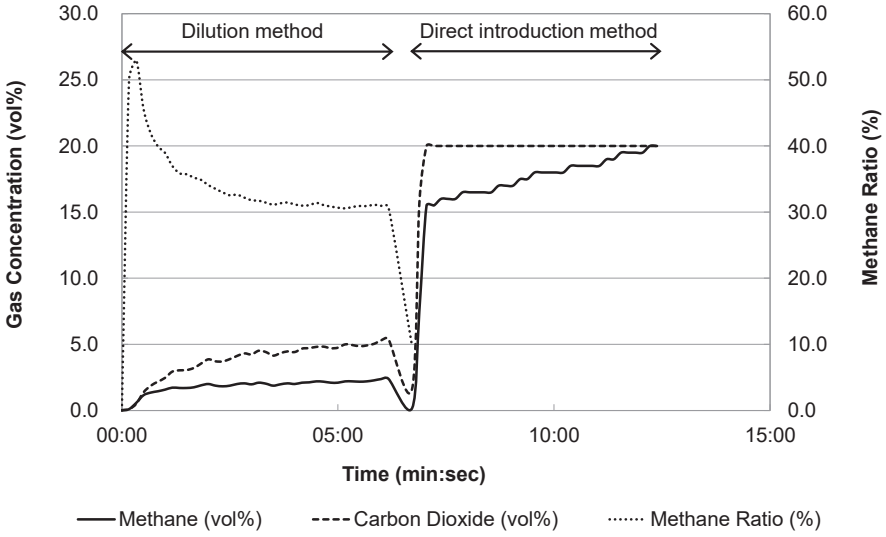

Fig. 2. Concentrations of methane and carbon dioxide, and the methane ratio in the rumen.
The time point at which rumen gas was introduced into the gas monitor was set to 0, and the dilution method was used until approximately 6 min and 30 s, after which the direct method was used until approximately 12 min and 30 s. The methane concentration is shown as a solid line, the carbon dioxide concentration is shown as a dashed line, and the methane ratio calculated from the data obtained from the dilution method is shown as a dotted line. Immediately after the start of the direct method measurement, the carbon dioxide level exceeded the upper detection limit of 20 vol%.

Table 5. Effect of spent coffee grounds (SCG) administration in term of methane ratio and related values.

	AM				PM			
	Before feeding		After feeding		Before feeding		After feeding	
Condition	Control	SCG	Control	SCG	Control	SCG	Control	SCG
	(n=8)	(n=6)	(n=8)	(n=6)	(n=8)	(n=6)	(n=8)	(n=6)
Methane Ratio (%)	51.8	42.3	58.6	54.2	52.1	36.6	51.6	48.1
	42.8	42.1	38.4	32.2	41.7	37.8	38.6	38.3
	42.9	42.8	36.6	31.6	36.7	41.1	31.6	45.8
	59.7	42.4	40.9	34.9	48.1	33.4	43.7	43.5
	68.8	47.2	41.6	30.0	52.6	40.8	41.7	34.7
	57.0	50.5	35.7	33.5	45.0	40.5	33.7	36.1
	46.3	-	33.4	-	42.0	-	32.0	-
	42.9	-	32.8	-	44.9	-	33.1	-
Median (%)	49.1	42.6	37.5	32.9	45.0	39.2	36.2	40.9
% Reduction	13.2		12.3		12.8		-13.1	
P-value	0.043		0.108		0.008		0.345	

n – number of reading
Experimental finding tabulated in term of methane ratio and its median. Facts about % reduction achieved in term of methane ratio and accordingly its impact on *P*-value have been demonstrated.

limit of 20 vol% immediately after switching to the direct method. The methane ratio calculated using the measured values from the dilution method continued to increase until approximately 3 min after the start of the measurements, then remained at equilibrium for approximately 2 min before the measurement method was switched.

Effect of spent coffee grounds supplementation on the methane ratio

Table 5 shows the methane ratios and the methane reduction rates. The median methane ratios before the morning feeding, after the morning feeding, before the afternoon feeding, and after the afternoon feeding were 49.1%, 37.5%, 45.0%, and 36.2%, respectively, in the control condition, showing a tendency to be higher before feeding and lower after feeding, and

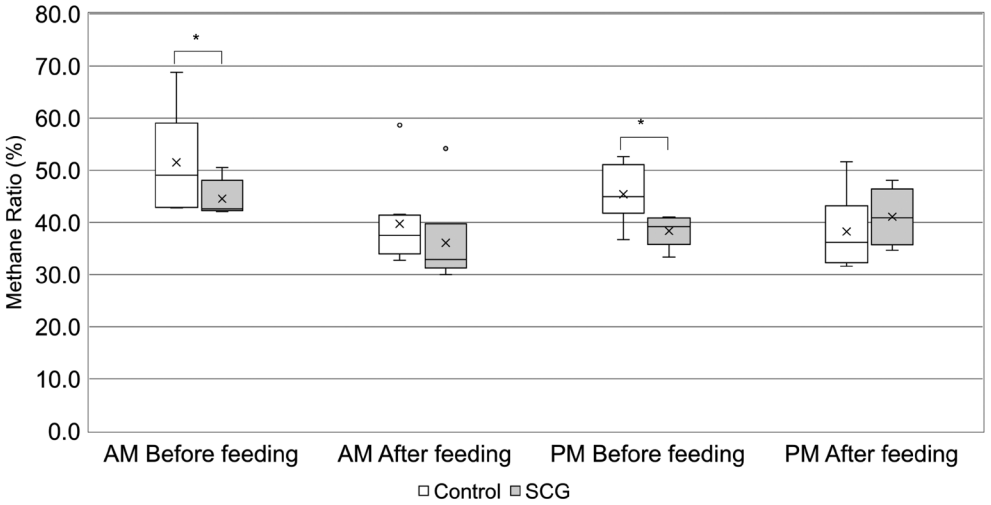

Fig. 3. Comparison of the methane ratios between the control and spent coffee grounds (SCG) conditions.
The notches on the box plots indicate the 95% confidence interval of the median, which was calculated as the median ± 1.58 × IQR / $\sqrt{(n)}$, with IQR being the difference between the third and first quartiles. In the figure, "×" indicates the mean of each sample, and "*" indicates a significant difference ($p<0.05$). SCG: spent coffee grounds; IQR: interquartile range.

42.6%, 32.9%, 39.2%, and 40.9%, respectively, in the SCG condition, showing the same tendency as in the control condition, except for after the afternoon feeding. Statistical analysis of the methane ratios in the control and SCG conditions at each measurement time showed that the methane ratio was significantly lower in the SCG condition than in the control condition before both the morning and afternoon feedings; however, no significant difference was observed in the methane ratios between the conditions after both the morning and afternoon feedings.

Discussion

Carbohydrates in the feed are broken down into their respective constituent sugars by the enzymes of rumen microorganisms. The sugars are further converted to pyruvate, which is metabolized into volatile fatty acids, such as acetic acid, propionic acid, and butyric acid, as well as gases, such as carbon dioxide and hydrogen (Knapp et al. 2014). In addition, formic acid is known to be formed in the rumen (Czerkawski 1986), and hydrogen is also produced by the decomposition of formic acid. Hydrogen produced in the rumen can interfere with the metabolic cycle if it remains in the rumen; however, the production of methane from hydrogen and carbon dioxide by methanogens reduces the hydrogen partial pressure in the rumen, thus reducing its effect on the metabolic cycle.

It has been reported that unsaturated fatty acids combine with the hydrogen produced in the rumen to form saturated fatty acids, thereby reducing the amount of methane produced in the rumen (Shiba et al. 2003). As shown in Table 4, the unsaturated fatty acids, oleic acid and linoleic acid, accounted for 50% of the fatty acids in SCG, suggesting that the unsaturated fatty acids in SCG combine with metabolic hydrogen in the rumen to suppress methane formation. Coffee beans are known to contain high levels of chlorogenic acid, an antioxidant (Socala et al. 2021), and as shown in Table 3, the chlorogenic acid remained in the SCG after extraction. It is presumed that the roasting of coffee beans partially oxidizes chlorogenic acid to chlorogenic acid quinone, which acts as a strong oxidant in the rumen, and that chlorogenic acid quinone is reduced to chlorogenic acid in the rumen when it reacts with hydrogen. The consumption of hydrogen in the rumen by this reaction is also considered to suppress the methanogenesis by methanogenic organisms.

We had expected the methane ratio in the SCG plots (Fig. 3) to be decreased both before feeding, when methane production in the rumen is at a minimum, and 3 h after feeding, when it is at a maximum; however, in this experiment, there was no decrease in the post-feeding methane ratio. The reaction in the mechanism described above occurs when the active ingredients in SCG, such as unsaturated fatty acids and chlorogenic acid quinone, come into contact with metabolic hydrogen. Compared to direct administration of the active ingredient, elution of the active ingredient from SCG in the rumen is considered to be relatively gentle, and it was assumed that it had no significant inhibitory effect on methanogenesis 3 h after feeding, when fermentation by methanogenic archaea actively occurs. On the other hand, before feeding in the morning and afternoon, when the amount of substrate is low and there is little fermentation by methanogenic archaea,

the degree of the changes in the methane level may have been larger, so the differences between the control and SCG conditions may have been more apparent even when the suppression effect on methane production was moderate.

In conclusion, by calculating the methane ratios from the methane and carbon dioxide concentrations measured by a rumen gas measurement system with a portable gas monitor, we found that the methane ratio was significantly lower in the SCG condition than in the control condition before the morning and afternoon feedings, and that methane production in the rumen was reduced in the SCG condition. Further elucidation of the detailed mechanisms is needed in the future. This measurement system utilizes an inexpensive gas monitor and can verify the methane reduction effect of feeds or feed additives, however, the dilution method has the limitation that it is difficult to determine total gas emissions and estimate accurate methane reductions on them. Future studies are necessary to develop an analysis method by comparing this approach with conventionally used measurement methods or using prediction equations for methane emissions.

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