Supplementation with whole cottonseed causes long-term reduction of methane emissions from lactating dairy cows offered a forage and cereal grain diet

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ABSTRACT

The objective of our work was to supplement a forage and cereal diet of lactating dairy cows with whole cottonseed (WCS) for 12 wk and to determine whether the expected reduction in CH₄ would persist. A secondary objective was to determine the effect of supplementing the diet with WCS on milk yield and rumen function over the 12-wk feeding period. Fifty lactating cows were randomly allocated to 1 of 2 diets (control or WCS). The 2 separate groups were each offered, on average, 4.2 kg of DM/cow per day of alfalfa hay (a.m.) and 6.6 kg of DM/cow per day of ryegrass silage (p.m.) on the ground in bare paddocks each day for 12 wk. Cows in each group were also individually offered dietary supplements for 12 wk in a feed trough at milking times of 5.4 kg of DM/cow per day of cracked wheat grain and 0.5 kg of DM/cow per day of cottonseed meal (control) or 2.8 kg of DM/cow per day of cracked wheat grain and 2.61 kg of DM/cow per day of WCS. The 2 diets were formulated to be similar in their concentrations of CP and ME, but the WCS diet was designed to have a higher fat concentration. Samples of rumen fluid were collected per fistula from the rumen approximately 4 h after grain feeding in the morning. Samples were taken from 8 cows (4 cows/diet) on 2 consecutive days in wk 2 of the covariate and wk 3, 6, 10, and 12 of treatment and analyzed for volatile fatty acids, ammonia-N, methanogens, and protozoa. The reduction in CH₄ emissions (g/d) because of WCS supplementation increased from 13% in wk 3 to 23% in wk 12 of treatment. Similarly, the reduction in CH₄ emissions (g/kg of DMI) increased from 5.1% in wk 3 to 14.5% in wk 12 of treatment. It was calculated that the average reduction in CH₄ emissions over the 12-wk period was 2.9% less CH₄ per 1% added fat, increasing

from 1.5% in wk 3 to 4.4% less CH₄ in wk 12. There was no effect of WCS supplementation on rumen ammonia-N, rumen volatile fatty acids, rumen methanogens, and rumen protozoa. On average over the 12-wk period, supplementation with WCS decreased the yield of milk (10%), fat (11%), protein (14%), lactose (11%), and fat plus protein (12%) and BW gain (31%). The WCS supplementation had no effect on milk fat concentration but resulted in a decrease in concentration of protein (5%) and lactose (11%). The major finding from this study is that addition of WCS to the diet of lactating dairy cows resulted in a persistent reduction in CH₄ emissions (g of CH₄/kg of DMI) over a 12-wk period and that these reductions in CH₄ are consistent with previous work that has studied the addition of oilseeds to ruminant diets.

Key words: dietary oil, methane emission, lactating dairy cow, sulfur hexafluoride tracer technique

INTRODUCTION

Agriculture was responsible for about 10 to 12% of total global anthropogenic emissions of greenhouse gases in 2005. Globally, emissions of methane (CH₄) and nitrous oxide (N₂O) increased by approximately 17% from 1990 to 2005. Enteric CH₄ fermentation accounted for about 32% of total non-CO₂ emissions from agriculture in 2005 (Smith et al., 2007).

Adding oils or oilseeds to the diet has been shown to be an effective means of mitigating CH₄ emissions of dairy cows (Martin et al., 2008; Beauchemin et al., 2009a). However, in recent reviews of adding lipids to the diet of ruminants, Beauchemin et al. (2009b) and Martin et al. (2010) pointed out that many of the studies have been short-term and that there is a need to demonstrate the long-term effect of oil-based feeding strategies.

Over the summer period in winter-rainfall dairying areas in Victoria, Australia, dryland pasture is generally of low availability and is also low in protein and energy content. Consequently, supplementary feeds often com-

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prise a large component of the diet, and sometimes the whole diet, and need to provide much of the energy and protein required by the animals. Oilseeds such as whole cottonseed (**WCS**) are high in energy and protein (14 MJ/kg of DM and 22% CP) and also contain about 22% oil. In a previous study, addition of 3 kg of WCS daily per cow over 5 wk to a diet of ryegrass silage, alfalfa hay, and cracked cereal grain reduced CH₄ emissions by 12% and increased milk yield by about 15% (Grainger et al., 2008). In the present work, rather than adding extra DM (energy) in the form of WCS to a basal diet of ryegrass silage, alfalfa hay, and cracked cereal grain, the 2 diets were formulated to be balanced for ME and CP. This was achieved by substituting cottonseed meal and extra cracked cereal grain in the control diet for WCS in the treatment diet. Consequently, the major difference between the 2 diets was the extra fat provided by the WCS.

The objective of our work was to extend the period of feeding WCS to dairy cows from 5 to 12 wk and determine whether the reduction in CH₄ would persist. A secondary objective was to determine the effect of supplementing the diet with WCS on milk yield and rumen function over a 12-wk feeding period.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

Fifty lactating cows, including 12 primiparous cows, from the experimental herd at the Department of Primary Industries–Victoria, Ellinbank Research Center (Ellinbank, Australia; 38°14' S, 145°56' E), 177 ± 10 DIM and with initial BW of 541 ± 56 kg, were allocated to 1 of 2 diets (control or WCS) so that the groups were balanced for means and variances of age, milk yield (measured during a covariate period 2 wk before treatments commenced), and calving date, as described by Baird (1994). Four of the cows in each group were ruminally fistulated. The experiment was conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (www.nhmrc.gov.au).

The 2 separate groups were each offered, on average, 4.2 kg of DM/cow per day of alfalfa hay (a.m.) and 6.6 kg of DM/cow per day of ryegrass silage (p.m.) on the ground in bare paddocks each day for 12 wk over summer and fall during 2008. The low levels of hay and silage offered per cow resulted in no recorded refusals of hay or silage for either group. Cows in each group were also individually offered dietary supplements for 12 wk in a feed trough at milking times. The control group was offered 6.0 kg of DM/cow per day of a mixture of cracked wheat grain (92%) and cottonseed meal (8%),

Table 1. Measured DMI of control and whole cottonseed (WCS) groups averaged over wk 1 to 12 of feeding of WCS¹

Item, kg of DM/cow per day	Control	WCS
Ryegrass silage	6.6 (0.6)	6.6 (0.6)
Alfalfa hay	4.2 (0.2)	4.2 (0.2)
Grain mixture ²	5.99 (0.12)	4.91 (0.82)
Total DMI	16.8 (0.6)	15.7 (0.6)

¹Data are means (SD). Standard deviation for silage and hay refers to variation in amounts offered per day to each group.

²Grain mixture for control diet comprised cracked wheat (92%) and cottonseed meal (8%). Canola meal was substituted for cottonseed meal in wk 11 of treatment because of a shortage of cottonseed meal. The WCS diet comprised cracked wheat (52%) and WCS (48%).

and the treatment group was offered 5.4 kg of DM/cow per day of a mixture of cracked wheat grain (52%) and WCS (48%; Table 1). The 2 supplement mixtures (wheat grain and cottonseed meal or wheat grain and WCS) were separately weighed for each cow and then thoroughly combined and offered to each cow as a mixture at milking times. Any refusals of the grain mixture were individually weighed, DM was measured, and the amount of each component was calculated based on the proportion of the wet weight of the individual components initially offered. The WCS was untreated and consisted of small seeds surrounded by lint. The 2 diets were formulated to be similar in their concentrations of CP and ME, but the WCS diet was designed to have a higher fat concentration (Table 2). Because of a shortage of cottonseed meal for the control diet, 0.9 kg of DM/cow per day of canola meal was used as a substitute for 0.5 kg of DM/d of the cottonseed meal in wk 11 of the treatment period to maintain the CP and ME contents of the control diet.

Cows were weighed fortnightly during the experiment and milked twice daily through a common parlor at about 0600 and 1500 h. Milk yield was measured daily for each cow using a DeLaval Alpro milk metering system (DeLaval International, Tumba, Sweden). One day per week a separate a.m. and p.m. milk sample was taken from each cow using in-line milk meters. Samples were analyzed for concentrations of fat, CP, and lactose using a near-infrared milk analyzer (model 2000, Bentley Instruments, Chaska, MN). Milk solids was defined as fat + protein.

Feed Analyses

Representative samples of feeds offered were collected each week, oven-dried and ground through a 0.5-mm sieve, and analyzed by a commercial laboratory (SGS Australia Pty Ltd., Toowoomba, Queensland). Some details of the methods used are given below. Fat content was analyzed by Soxhlet extraction using petro-

Table 2. Nutrient composition of the feeds and diets, control and whole cottonseed (WCS), offered over wk 1 to 12 of feeding of WCS

Item, g/kg of DM unless noted	Ryegrass silage	Alfalfa hay	Wheat	Whole cottonseed	Cottonseed meal	Canola meal	Diet	
							Control (average)	WCS (average)
DM	41.7	83.0	89.8	91.6	89.0	88.4	—	—
CP	200	198	156	226	508	296	194	196
Fat	32	8	14	232	19	6.4	19.7	52.6
NDF	566	462	153	581	176	241	390	469
ME, ¹ MJ/kg of DM	10.1	10.0	13.3	15.1	11.4	11.8	11.2	11.4

¹Calculated according to NRC (1984) except for whole cottonseed, which was calculated according to NRC (2001).

leum spirit with breakdown of lint for whole cottonseed (AACC method 30–20, AOCS Aa 4–38; AOCS, 1997). Ryegrass silage, alfalfa hay, and canola meal were analyzed by near-infrared spectroscopy for CP, crude fat, NDF, and ME. Whole cottonseed and cottonseed meal were analyzed for protein by Dumas combustion using a Leco TruSpec N nitrogen analyzer (Leco Corp., St. Joseph, MI) and for NDF by Van Soest extraction (Van Soest et al., 1991) using the Foss FiberCap technique (Foss Tecator, 2002). The ME concentration of the WCS was based on analysis by near-infrared spectroscopy by a different commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY) using a calculation based on NRC (2001). Analytical composition of feeds is shown in Table 2.

Methane Emissions

Measurements of CH₄ emissions using the sulfur hexafluoride (SF₆) tracer gas technique were made on 12 cows from each dietary group on 3 consecutive days in wk 2 of the covariate period and wk 3, 6, 10, and 12 as described by Grainger et al. (2007). Permeation tubes had been placed in the rumen of each cow by way of the mouth 154 d before the first SF₆ measurements made during the covariate period.

Ruminal Fermentation, Methanogens, and Protozoa

Samples of rumen fluid were collected per fistula from 4 locations in the rumen (in the fiber mat, below the fiber mat, anterior to the fiber mat, and midway down the posterior end of the fiber mat) approximately 4 h after grain feeding in the morning. Samples were collected from 8 cows (4 per diet) on 2 consecutive days in wk 2 of the covariate and wk 3, 6, 10, and 12 of treatment. The rumen fluid samples were analyzed for VFA, ammonia-N, methanogens, and protozoa. For VFA, 4 mL of rumen fluid were pipetted into a tube containing 1 mL of 25% metaphosphoric acid and fro-

zen for subsequent analysis, based on the method of Erwin et al. (1961), on a gas chromatograph (Varian CP-3800, Varian, Palo Alto, CA) using a polar column (AT-1000 stationary phase, 0.20 µm film thickness, 25 mm length, 0.25 mm i.d.; Alltech Capillary Column, Alltech, Lexington, KY) and a flame ionization detector. For ammonia-N, 10 mL of rumen fluid was pipetted into a tube containing 10 mL of 0.1 M HCl and frozen for subsequent analysis. Prior to analysis, 10 mL of the preserved sample was further diluted with 20 mL of the 0.1 M HCl and then centrifuged at 1,780 × *g* for 15 min. Supernatant was collected and the pH was adjusted with concentrated NaOH to pH >12.5. Ammonia concentration was measured with an ion selective electrode (TPS uniPROBE ammonia ion selective electrode, part number 121700; Thermal model 9512 ammonia gas sensing electrode, Thermo Electron Corp., Beverly, MA, used in conjunction with a TPS model 440 lab analyser, TPS, Springwood, Queensland, Australia) calibrated to standard solutions of 1 and 25 mg/L of NH₃.

For methanogens and protozoa, the rumen fluid was squeezed through a 1-mm plastic sieve and 1 mL of well-mixed fluid was transferred into a 10 mL centrifuge tube (4 samples per cow) and spun at 3,645 × *g* for 5 min. The supernatant was decanted and the pellet was frozen for subsequent analysis. Using the cetyltrimethylammonium bromide (CTAB) method of Wright et al. (1997), DNA from rumen contents of individual animals was extracted. Each DNA sample was analyzed using real-time PCR to determine the density of methanogens and protozoa in the extracted DNA. For each microbial group (methanogenic archaea and ciliate protozoa), 3 different sample aliquots of DNA were analyzed using real-time PCR as described previously by Sundset et al. (2009) using the rumen protozoal 18S rRNA gene primers P-SSU-316f and P-SSU-539r (Sylvester et al., 2004) and the methanogen methyl-coenzyme M reductase subunit primers qmcrA-F and qmcrA-R (Denman et al., 2007). The external standards used for the real-time

PCR amplifications have been validated previously for ciliate protozoa (Skillman et al., 2006) and methanogenic archaea (Denman et al., 2007).

Statistical Analyses

Daily milk yield data ($n = 25$ cows/diet), including the 2-wk covariate period, were analyzed using a generalized mixed model that was defined with fixed factorial effects for treatment by parity (cow/heifer) by week, and a spline to account for the general changes in yield with time. The spline smoothing parameter was estimated as a random effect. Additional random effects were specified for day, random linear coefficients over time for each cow, and an order 1 auto-regressive process over day within cows. Analysis was conducted using REML software GenStat (2008).

Fat, protein, solids (fat + protein), and lactose percentages and yields, measured fortnightly, were analyzed using a split-plot analysis of covariance (ANCOVA; animal split for date, with the corresponding data collected in the 2 wk before the experiment started as the covariate). The treatment structure was factorial treatment by parity by week. Changes in BW over the treatment period were analyzed by simple ANOVA with cow as the experimental unit. Analyses were conducted using ANOVA software (GenStat, 2008).

Methane data ($n = 12$ cows/diet) were analyzed using a split-split-plot ANCOVA (animal split for week split for day, with the corresponding data collected in the 1 wk before the experiment started, averaged over days, as the covariate). The treatment structure was factorial WCS treatment by parity by week. Analyses were conducted using ANOVA software (GenStat, 2008). Residuals from each analysis were checked graphically for constant variance and normality of distribution.

For methanogen and protozoa data ($n = 4$ cows/diet), after checking that duplicate data (consecutive days) were consistent, the data were $\log_{10}$ -transformed and averaged within cows, and then analyzed using ANCOVA with the corresponding data for the covariate period as the covariate. *P*-values were derived using (nonparametric) ANCOVA permutation tests. Analyses were conducted using ANOVA software (GenStat, 2008).

RESULTS

Feed Intakes and Diet Composition

The DMI of alfalfa hay and ryegrass silage for each dietary group were separately measured as an average of the total amount fed to each group over wk 1 to 12 (Table 1). Because there were no refusals of hay

or silage, the amounts offered were assumed to be the amounts eaten. The wheat grain, cottonseed meal, canola meal, and WCS were weighed on an individual cow basis and there were minimal refusals, except for WCS, so the intakes presented in Table 1 are accurate. The thorough mixing of the grain supplements meant that there was limited opportunity for cows to sort the individual components (wheat from either cottonseed meal or whole cottonseed). Some of the cows refused the WCS-crushed wheat mixture, resulting in an average WCS intake for the group of 2.35 kg of DM/cow per day and an average intake of crushed wheat of 2.56 kg of DM/cow per day. On average over the 12-wk treatment period, 6 of the 25 cows receiving WCS refused >0.5 kg of DM/d, and the maximum amount refused by a cow over the 12-wk period was 0.98 kg of DM/d. The average total DMI for the control group during wk 1 to 12 was 1.1 kg of DM/cow per day higher than for the WCS group. The average weekly total DMI over the 12-wk treatment period ranged from 16.3 to 18.1 kg of DM/d for the control group and from 15.0 to 16.8 kg of DM/d for the WCS group. The WCS eaten over the 12 wk ranged from 2.12 to 2.54 kg of DM/d.

The concentration of CP and ME was similar for the 2 diets as expected, but the NDF concentration of the WCS diet was about 80 g/kg of DM higher than the control diet (Table 2). The fat concentration in the WCS diet was 33 g/kg of DM higher than in the control diet.

Methane Emissions

The reduction in CH_4 emissions (g/d) because of WCS supplementation increased from 13% in wk 3 to 23% in wk 12 of treatment (Table 3). Similarly, the reduction in CH_4 emissions (g/kg of DMI) increased from 5.1% in wk 3 to 14.5% in wk 12 of treatment. In contrast, there was no significant interaction (treatment $\times$ wk) for CH_4 emissions (g/kg of milk) and the average reduction over the 12 wk was 9%. It was calculated that the average reduction in CH_4 emissions over the 12-wk period was 2.9% less CH_4 per 1% added fat, increasing from 1.5% in wk 3 to 4.4% less CH_4 in wk 12.

Milk Yield and BW Change and Rumen Fluid Parameters

Milk solids yield exhibited an interaction of treatment $\times$ week ($P < 0.01$) because of a greater decline in yield for cows fed WCS throughout the 12-wk experiment compared with those on the control diet (Figure 1). On average over wk 1 to 12 of treatment, supplementation with WCS decreased the yield of milk (10%), fat (11%), protein (14%), lactose (11%), and milk solids (12%) and

BW gain (31%; Table 4). The WCS supplementation had no effect on milk fat concentration but resulted in a decrease in concentration of CP (5%) and lactose (11%). There were no effects of treatment or treatment $\times$ week observed for ruminal fluid variables, so only main effects are reported (Table 5).

DISCUSSION

The major finding from this study is that addition of WCS to the diet of lactating dairy cows resulted in a persistent reduction in CH_4 emissions of 3.5 g of CH_4 /kg of DMI over a 12-wk period. The reduction in CH_4 emissions increased from 1.5% during wk 3 to 4.4% per percentage unit of added lipids during wk 12 of treatment. The cows in our experiment refused, on average over the 12-wk period, 0.35 kg of DM/d of WCS (weekly average ranged from 0.12 to 0.54 kg/d), but the greatest refusals appeared to be during periods of hot weather ($>30^\circ\text{C}$) and were not related to the amount of time on treatment and therefore cannot explain the greater reduction in CH_4 emissions over time. These reductions in CH_4 are consistent with previous work that has studied the addition of oilseeds to ruminant diets (Martin et al., 2010).

The persistent effect of oils on CH_4 was also observed by Jordan et al. (2006), who offered 8% refined coconut oil to crossbred beef heifers over a 93-d period. In that study, CH_4 emissions were measured twice over 5-d

Table 3. Effects of whole cottonseed (WCS) on methane production in wk 3, 6, 10, and 12 of feeding WCS ($n = 12$ per diet)^{1,2}

Item	Diet		LSD	P-value
	Control	WCS		
Methane, g/d			39.4	0.011
Wk 3	592	512		
Wk 6	600	505		
Wk 10	600	505		
Wk 12	625	480		
Average, wk 1–12	604	501		
Methane, g/kg of milk			2.83	0.048
Average, wk 1–12	33.3	30.4		
Methane, g/kg of DMI			3.30	0.048
Wk 3	35.4	33.6		
Wk 6	36.3	32.8		
Wk 10	36.2	32.8		
Wk 12	37.2	31.8		
Average, wk 1–12	36.3	32.8		

¹Values are predicted means adjusted for methane production in wk 2 of the covariate period.

²The LSD and P-values are presented for the interaction term (treatment $\times$ wk) except for methane (g/kg of milk) when the interaction term was not significant ($P = 0.76$) and values for the main treatment effect are presented.

Figure 1. Trend in milk solids (fat + protein) yield (mean $\pm$ 95% confidence limits) from the 2-wk covariate period (wk -2, -1) and over the 12-wk treatment period for cows receiving a control diet ($\blacktriangle$) and cows receiving a whole cottonseed (WCS) diet ($\blacksquare$). Interaction term (treatment $\times$ week) was significant ($P < 0.001$; LSD = 0.048). The control diet comprised ryegrass silage, alfalfa hay, cracked wheat grain, and cottonseed meal. The WCS diet comprised ryegrass silage, alfalfa hay, cracked wheat grain, and WCS.

periods at 14 and 70 d after treatment started. There was no significant interaction (treatment $\times$ time) so the main effect was reported, which was a 19.8% reduction in CH_4 (L/kg of DMI) because of coconut oil, averaged over the 2 periods.

Two other experiments have studied the long-term effect of adding oils to dairy cow diets (Johnson et al., 2002; Woodward et al., 2006) and in both cases it was concluded that there was no persistent effect of oils on CH_4 emissions. In the experiment of Johnson et al. (2002), CH_4 emissions were estimated every 3 mo during lactation from 2 replicates of 4 cows using a room tracer approach. Their experimental design would be unlikely to have great power in detecting effects of treatment on CH_4 emissions because of a low number of degrees of freedom. The diet $\times$ time interaction was not significant. There was no effect on CH_4 emissions of adding a medium level of 1.7% fat to a TMR diet measured as grams per day, but when expressed per kilogram of FCM, the CH_4 emissions tended to be lower. Adding 1.7% fat to the diet increased total DMI by 2 kg/d; this would be expected to result in increased emissions (Grainger et al., 2007), but this was not considered in their paper. If the CH_4 emissions are expressed per kilogram of DMI, then there is a 5.8% reduction in emissions (15.4 vs. 14.5 g of CH_4 /kg of DMI). This can then be expressed as a 3.4% decrease in CH_4 per 1% added fat, a value consistent with other reported data (Martin et al., 2010). In the experiment of Johnson et al. (2002), an additional 1.6% fat was added to the diet, bringing the total amount of fat in the diet to

Table 4. Effects of whole cottonseed (WCS) on yield of milk, milk composition, and BW change averaged over 12 wk (n = 25 cows/diet)

Item	Diet		LSD	P-value
	Control	WCS		
Milk yield, kg/d	18.1	16.2	0.56	<0.001
Fat, %	5.01	4.94	0.142	0.33
Fat yield, kg/d	0.92	0.81	0.038	<0.001
CP, %	3.46	3.30	0.052	<0.001
Protein yield, kg/d	0.64	0.55	0.017	<0.001
Lactose, %	4.90	4.82	0.033	<0.001
Lactose yield, kg/d	0.91	0.81	0.032	<0.001
Milk solids yield, ¹ kg/d	1.55	1.36	0.048	<0.001
BW change wk 1–12, kg/d	0.48	0.33	0.110	0.01

¹Milk solids are milk fat plus protein.

5.6% (high fat diet). This extra fat produced total DMI and 3.5% FCM that were similar to the medium fat diet but a lower milk protein percentage. However, the CH₄ emissions from this high fat diet were numerically higher than for the control diet, a result that is inconsistent with the literature and that was not explained by the authors.

Woodward et al. (2006) reported on a 12-wk study in which cows grazing pasture were drenched with 300 g/d of a mixture of linseed and fish oil. Measurements of CH₄ were made using the SF₆ tracer technique during wk 12 of treatment. There was a 5.8% reduction in emissions (23.0 vs. 21.7 g of CH₄/kg of DMI) because of oil, but this was not significant (a difference of 9.2% could have been detected as significant). However, the measured difference is a 3.1% reduction in CH₄ per 1% added fat, a value consistent with data from our paper and Johnson et al. (2002). Therefore, it appears that the experimental design used by Woodward et al. (2006) was not sensitive enough to detect the expected reduction in CH₄ emissions. Data from at least 4 experiments (this experiment; Johnson et al., 2002; Jordan et al., 2006; and Woodward et al., 2006) are quite consistent in the magnitude of the effect of oils in reducing CH₄ emissions and indicate a reduction in CH₄ that persists over the long-term.

Condensed tannins (CT) are phenolic compounds that have been shown to reduce enteric CH₄ emissions from ruminants (Waghorn et al., 2002). Grainger et al. (2009) have shown that addition of 266 g of CT from *Acacia mearnsii* to a basal diet of pasture was associated with a 126 g/d reduction in CH₄ emissions. Assuming that the cottonseed meal used in this experiment contains approximately 1.6% CT (Terrill et al., 1992) and that the CT from cottonseed meal has the same antimethanogenic potential as the CT from *A. mearnsii*, we calculate that the difference in CT content between the control and WCS diets would have been

approximately 30 g of CT (1.87 kg × 16 g/kg); this could have accounted for a reduction in CH₄ emissions of approximately 8 g/d. In this experiment the WCS diet contained significantly more NDF than the control diet. Furthermore, WCS generally has a low concentration of starch (<10% DM) whereas wheat generally has a high concentration (>50% DM). Diets with high fiber and low starch are generally considered to result in higher enteric methane emissions than low fiber, high starch diets (Beauchemin et al., 2008). Taking into account the above considerations, in this experiment the reduction in CH₄ emissions associated with feeding the WCS diet can be attributed almost entirely to the lipid content of the WCS diet.

In the present study the average values for CH₄ emissions over the 12-wk experimental period were 36.3 and 32.8 g of CH₄/kg of DMI for the control and WCS diets, respectively; these values are high relative to other published data. In most studies, measurements of CH₄ emissions made by the SF₆ technique are of similar magnitude to measurements of CH₄ emissions made using respiration chambers (Johnson et al., 1994; Boadi et al., 2002; McGinn et al., 2006; Grainger et al., 2007; Pinares-Patiño et al., 2008). However, there are some cases where this is not true. For example, Pinares-Patiño et al. (2008) measured CH₄ emissions of sheep using both chambers and the SF₆ technique and measured a 39% higher CH₄ emission using the SF₆ technique compared with the chamber. The permeation tubes used in their experiment had been placed in the rumen 250 d before the SF₆ measurements were made. Unpublished data was also available from 4 other experiments conducted at our center, and in 2 of these experiments permeation tubes had been placed in the

Table 5. Effects of whole cottonseed (WCS) on rumen fluid ammonia-N, VFA, rumen methanogens, and rumen protozoa (n = 4 cows/diet)¹

Item	Diet		LSD	P-value
	Control	WCS		
Ammonia-N, mg/L	298	259	55.6	0.90
Total VFA, mmol/L	114.2	117.0	16.16	0.72
VFA, mol/100 mol				
Acetate	66.0	64.8	9.93	0.61
Propionate	17.7	17.2	2.58	0.53
Butyrate	12.6	14.4	2.28	0.89
Rumen methanogens				
×10 ⁷ /mL	3.72	2.34	NA ²	NA
log ₁₀ value	7.57	7.37	0.543	0.40
Rumen protozoa				
×10 ⁷ /mL	6.37	2.74	NA	NA
log ₁₀ value	6.80	6.44	0.495	0.17

¹Rumen fluid was taken 4 h after feeding.

²NA = not applicable because the statistical analysis was conducted on log₁₀ data.

rumen about 150 d before the SF₆ measurements were made. Combining the data from the 5 published experiments already cited with the unpublished data from 4 other experiments from our research center enabled a positive relationship to be established between the duration that a permeation tube resides in the rumen before measurements are made and the discrepancy between the CH₄ emission as estimated by the SF₆ and chamber techniques:

$$Y = 98.0(\pm 2.7) + 1.3(\pm 0.2)X,$$

where $Y = \text{CH}_4$ estimated by SF₆/CH₄ estimated by chamber, expressed as a percentage, and $X = \text{weeks to first SF}_6 \text{ measurement post permeation tube insertion}$ ($R^2 = 0.89$, $n = 9$ experiments).

Using this relationship, it can be calculated for our experiment that the SF₆ technique would overestimate the chamber technique by 27%. If this 27% correction is applied to our CH₄ data, then the value would be 26.5 g of CH₄/kg of DMI for the control group and 23.9 g of CH₄/kg of DMI for the WCS group, which would be more comparable with other reported data. Although the CH₄ reported here may be high as an artifact of the length of time the permeation tubes were in the rumen, the relative differences between treatments and longevity of effect of WCS remains valid.

Adding lipids to the diet is known to reduce protozoal and methanogen numbers in the rumen (Yang et al., 2009; Martin et al., 2010). Beauchemin et al. (2009a) measured a significant reduction in protozoal numbers when adding sunflower (38%) or canola (37%) seeds to the diet of lactating dairy cattle, and this could have explained a large part of their measured reduction in CH₄ emissions. In our experiment protozoal numbers tended to decline (−57%) more than that measured by Beauchemin et al. (2009a), but the sensitivity of our experimental design enabled us to detect a 68% reduction in protozoa numbers. Ivan et al. (2004) measured a 43% decrease in rumen ammonia-N associated with reduced protozoal numbers and our data tend to support this observation, but the measured decrease in our work was only 13%, which was not significant. Methanogens tended to decline (−37%) in our experiment by adding WCS to the diet, but our experimental design enabled us to detect a 71% reduction in methanogen numbers. Even though the addition of WCS to the diet tended to reduce protozoal and methanogen numbers and rumen ammonia-N in the rumen fluid, the design of our experiment was not powerful enough to detect these effects. In future work, there needs to be a greater number of animals involved to measure the effects of

treatments on rumen fluid parameters if significant differences are to be obtained.

Supplementation of the diet with WCS resulted in an average reduction of 1.9 kg of milk/cow per day over the 12-wk period compared with the control group. This was unexpected and was mainly because of the decline in ME intake of the WCS group. The reduced ME intake was caused by refusals of the WCS-cracked wheat mixture by the cows in the WCS group. The refusals of the grain mixture appeared to be greater in hot weather (>30°C), but there was always some refusal of the mixture. In a review of WCS in dairy cattle feeding, Arieli (1998) concluded that DMI is not altered when WCS is included at up to 25% of the diet, but that under hot weather conditions combined with a higher fiber content of the WCS diet, a decline in DMI occurred with WCS feeding. The decline in DMI observed in our experiment may be explained by the fact that our experiment was conducted in late summer, during which there were more than 5 hot (>35°C) days, and the fact that the WCS diet had a higher NDF content than the control diet (469 vs. 390 g/kg of DM). Arieli (1998) also concluded that under hot weather conditions where both the control and WCS diets had a similar fiber level, there was no decline in intake of the WCS-supplemented diet. This agrees with previous work at our center with WCS feeding (Grainger et al., 2008) where there were hot weather conditions but the NDF levels of the WCS and control diets were similar (428 and 415 g of NDF/kg of DM, respectively), and there was no refusal of WCS. This suggests that when diets are supplemented with WCS under hot weather conditions it is important to ensure that there is no increase in the fiber content of the diet; otherwise, DMI and milk yield will probably decline.

We conclude that the addition of WCS to the diet of lactating dairy cows resulted in a persistent reduction in CH₄ emissions (g of CH₄/kg of DMI) over a 12-wk period and that these reductions in CH₄ are consistent with previous work that has studied the addition of oilseeds to ruminant diets.

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