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Research Article

Dairy Cow Feed Supplementation Alternatives for Diminishing Methane and Carbon Dioxide Concentration *In Vitro*

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Abstract: The objective of this study was to evaluate the effect of five different supplementations [Yeast-*Saccharomyces cerevisiae* (YS), tea leaves-*Camellia sinensis* (TL), Red Sorghum (RS), soybean oil (SBO) and Chinese soapberry - *Sapindus mukorossi* (CS)] on the reduction of methane (CH_4) *in vitro* conditions. Tests were done by adding each of the 5 supplementations (0.005 g X1010 for YS; 5 g for TL, RS and CS; and 5 mL for SBO) to the ruminal liquor (1.5 L/digester) of a cow fed Total Mixed Ration and incubated for 24 h. Results show that all the supplementations significantly decreased CH_4 concentration compared to the control treatment ($p<0.05$). *In vitro* CH_4 concentration was reduced from 56.75 to 11.14, 13.32, 19.88, 20.07%, respectively and 30.52% for SBO, TL, RS, CS and YS, respectively. CH_4 concentration tended to be higher during the first hours of incubation in the control treatment compared to all supplementations. However, the supplementations reduced CH_4 concentration during the first part of the incubation. This demonstrates that the supplementations used in this experiment are very effective to reduce CH_4 concentration as early as 2 h after supplemented. Nevertheless, no significant interactions between treatments × time were found in CH_4 concentration ($p>0.001$). CH_2 concentration was significantly higher for the control treatment (1769.07 ppm) and YS (1683.41 ppm) compared to TL (1342.98 ppm), CS (1173.61 ppm), RS (1193.39 ppm) and SBO (991.25 ppm) supplementation ($p<0.05$). All the supplementations, but especially SBO, have the potential to diminish greenhouse gas production *in vivo* and may be alternatives for the mitigation of global warming.

Keywords: Dairy cow (*bos taurus*), greenhouse gas, global warming, soybean oil, tea (*camellia sinensis)*

INTRODUCTION

Global warming, caused by the increasing concentration of greenhouse gases (GHG), is one of the most significant threats facing our world today. GHG is caused by the buildup of gases that trap heat in the atmosphere. According to Milich (1999), these gases are largely anthropogenic in origin and are now at greater concentrations than at any time in the past 160,000 Yrs.

Carbon dioxide (CO_2) is the principal GHG, followed by methane (CH_4) and nitrous oxide (N_2O). However, the global warming potential of CH_4 is 23-25 times that of CO_2 (Christophersen *et al*., 2008). Most of the CH_4 produced within agricultural systems comes from ruminants. CH_4 is a natural by-product of animal digestion through a process known as enteric fermentation. This fermentation leads to an inefficient use of energy from feed and causes ecological problems. The amount of CH_4 produced depends on the type of animal and the amount and kind of feed it consumes, among other variables (Kinsman *et al*., 1995).

The CH_4 atmospheric lifetime is relatively short (≈10yrs) compared to that of CO_2 (≈100+yrs) (Boucher *et al*., 2009). Thus, reducing CH_4 emissions would have a more immediate and significant impact on mitigating climate change than just reducing CO_2 emissions. For this reason, there is a worldwide renewed interest in finding sustainable feeding strategies for reducing emissions of CH_4 from ruminants (Jarvis *et al*., 1995). Several methods have been proposed for CH_4 mitigation, i.e., utilization of condensed tannins (Puchala *et al*., 2005; Ramírez-Restrepo and Barry, 2005), saponins (Lila *et al*., 2003 and Wina *et al*., 2005) and oil extracted from various plants (Johnson *et al*., 2002; Jordan *et al*., 2006b). However, most of the studies are for only one supplementation at a time, which affects the possibility of comparing effectiveness among treatments. The supplementation of yeast, on the other hand, has been used to enhance nutrition and health in dairy cows, but little research has been done about its effects on reducing CH_4. This research intends to compare different feed supplements such as oil, yeast and others containing secondary compounds (i.e.,

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Table 1: Composition of TMR fed to milking cows

Ingredients	Proportion (%)*
Napier grass (*Pennisetum purpureum*)	42.75
Concentrate	14.23
Silage	42.75
$NaHCO_3$	0.35

*19% DM basis

Table 2: Concentrate composition included in the TMR

Ingredient	(%)
Corn meal	65
Soybean meal	15
Cracked corn	5
Wheat brand	10
Tallow	0.5
Ca	2.0
NaCl	1.0
NaHCO3	1.0
Premix	0.5
Chemical Composition	
DM	89%
CP	16.25%
ME	3 Mcal/kg
NEL	1.95 Mcal/kg
NDF	15.80%
NFC	60%
FAT	3.75%
UIP	35%

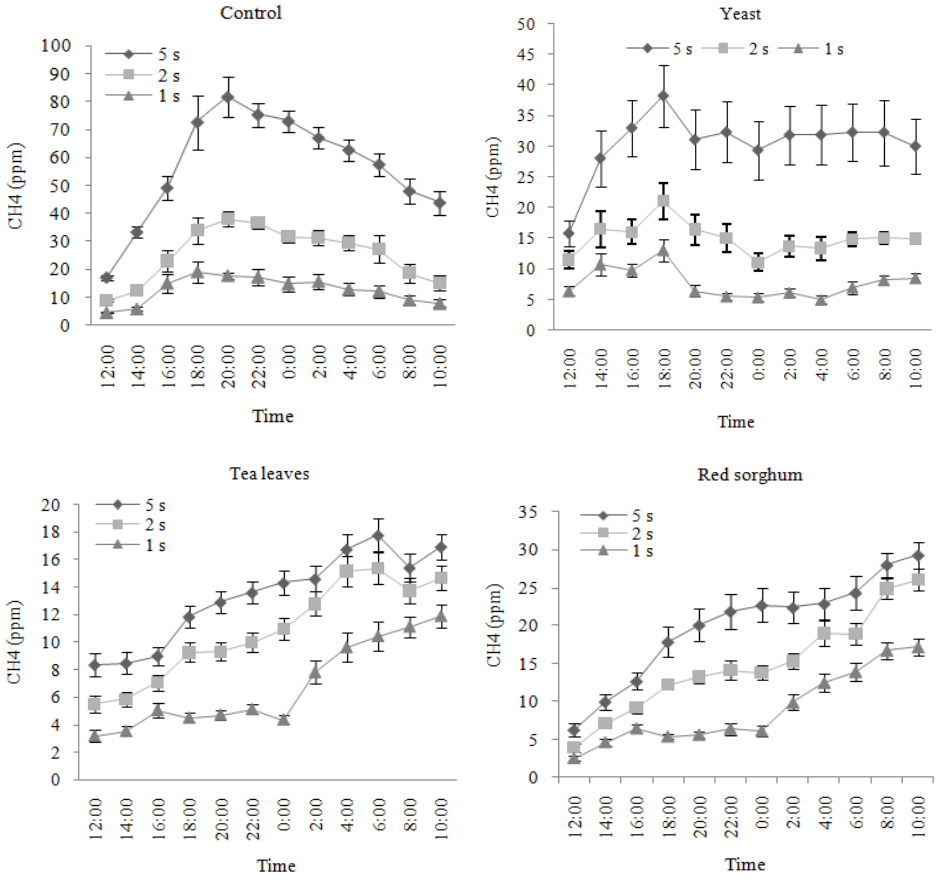
DM: Dry Matter; CP: Crude Protein; ME: Metabolizable Energy; NEL: Net Energy of Lactation; NDF: Neutral Detergent Fiber; NCF: Non-fiber carbohydrate; UIP: Undegradable Intake Protein

tannins, saponins) that might be used in ruminants' diets to diminish GHG.

MATERIALS AND METHODS

Treatments: Five different supplementations [LEVUCELL SC20® yeast- *Saccharomyces cerevisiae* (YS), Tea leaves-*Camellia sinensis* (TL), Red Sorghum (RS), soybean oil (SBO) and a byproduct of Chinese soapberry-*Sapindus mukorossi* (CS)] were added to compare their effect on the concentration of CH_4 *in vitro*. The amount of each supplementation was 0.005 $g\times10^{10}$ for YS, 5 g for TL, RS and CS; and 5 mL for SBO. All these supplementations were compared to a control, where no supplementation was added. The amount of supplement included in every treatment was determined after a pre-trial. Food was provided to a cow with different amounts of the supplements every day. Since adding the supplements affected the palatability, the ideal dose of supplementation should be the maximum amount that does not severely compromise the cow's feed intake.

Ruminal liquor collection and preparation: The protocol for this research was approved by the Innovation and Practical Training Center, National Pingtung University of Science and Technology. Ruminal liquor was collected from 1 cannulated Holstein cow. The cow was fed *at libitum* with Total Mixed Ration (TMR) (Table 1 and 2) and had access to fresh water at all times. Fresh feed was provided



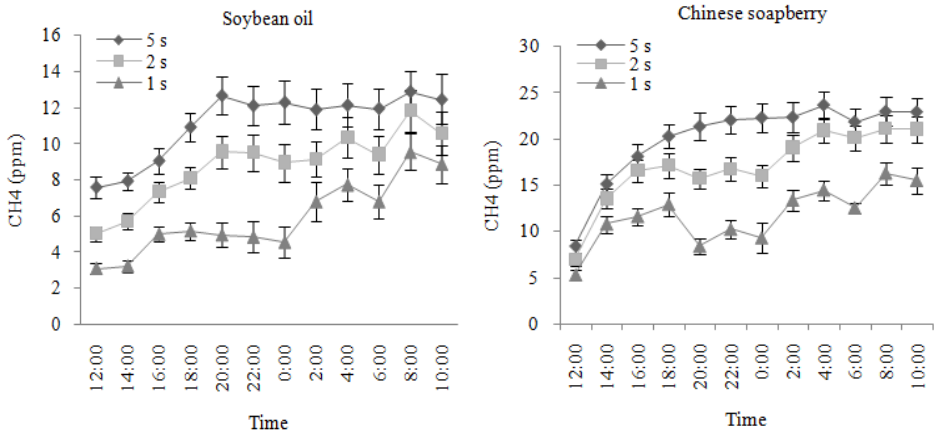


Fig. 1: Interaction between treatment and time on CH_4 concentration at 1, 2 and 5 s after opening the valve ($p>0.001$)

Table 3: CH_4 and CO_2 concentrations for five different supplementations

GHG	Time	Control	YS	TL	RS	SBO	CS
CH_4	1s	12.66±0.55^a	7.64±0.34^d	6.78±0.24^e	8.95±0.34^c	5.86±0.25^f	11.68±0.33^b
(%)	2s	25.59±0.88^a	14.94±0.62^c	10.80±0.29^d	14.83±0.45^c	8.76±0.28^e	17.02±0.40^b
	5s	56.75±1.67^a	30.52±1.35^b	13.32±0.30^d	19.88±0.63^c	11.14±0.31^e	20.07±0.45^c
CO_2	1s	503.17±5.20^d	638.19±3.31^a	590.07±3.17^c	616.39±2.30^b	592.84±3.10^c	631.92±1.98^a
(ppm)	2s	776.24±13.74^a	761.75±5.04^b	718.71±5.14^c	719.92±4.69^d	663.08±4.01^e	751.41±3.47^d
	5s	1668.21±11.78^a	1211.33±10.10^a	1096.58±11.92^b	1059.43±7.69^c	931.68±7.23^d	1097.36±6.60^c

Means followed by different letters are significantly different at Duncan's multiple range test ($p<0.05$); YS: Yeast supplementation; TL: Tea leaf supplementation; RS: Red sorghum supplementation; SBO: Soybean oil supplementation; CS: Chinese soapberry; MSE: Mean squared error

twice a day at 06:30 and 18:30. Ruminal liquor was collected 3 h after feeding (09:30). The ruminal fluid was then strained through 2 layers of gauze before placing it in the digester (Fig. 1).

Equipment: A BioFlo® 110 Fermentor/Bioreactor was used in the experiment to simulate the rumen conditions. Four digesters (glass vessels) were filled with 1.5 L of ruminal liquor. The pH was set to 6.4 and was automatically controlled with sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH). The temperature was kept stable at 39°C with a heater blanket. The movement conditions of the rumen were simulated with a pump at 150 rpm. CO_2 was flushed in each digester for 2 sec to establish anaerobic conditions (Fig. 2).

Analysis: Thirty two replications were done, with a total of 2304 observations. Every incubation was done during a period of 24 h. CH_4 concentration (%) was measured every 2 h during 24 h of observations. CH_4 measurements were done using a VRAE multigas monitor (PGM-7840) and CO_2 concentration measurements (ppm) were done with an ALNOR Compu Flow CO_2 Meter at the 1st, 2nd and 5th second of opening the valve.

Statistical analysis: The data from the experiment was subjected to a General Lineal Model (GLM) and Analysis of Variance (ANOVA). The means were later compared for significance using Duncan's test at $p<0.05$ (SAS, 2000).

RESULTS

In vitro CH_4 concentration was significantly lower for all 5 supplementations when compared to the control treatment (Table 3). CH_4 concentrations were reduced by 80% for SBO, 77% for TL, 65% for RS, 64% for CS and 46% for YS ($p<0.05$). CH_4 concentration tended to be higher during the first hours of incubation in the control treatment. However, the supplementations reduced CH_4 concentration during the first part of the incubation. This demonstrates that the treatments used in this experiment were effective in reducing CH_4 concentration as early as within 2 h after supplementation. However, no significant interactions between treatments× time were found in CH_4 concentration ($p>0.001$) (Fig. 1).

CO_2 production was significantly higher in the control treatment and YS supplementation compared to TL, CS, RS and SBO supplementations ($p<0.05$). However, SBO supplementation represented the most important decrement in CO_2 concentration (43%) compared to the control treatment (Table 3). No significant interactions between treatments×time were found in CO_2 concentration ($p>0.001$) (Fig. 2).

DISCUSSION

Adding oil to ruminants' diets has been shown to decrease CH_4 not only by lowering ruminal substrate fermentability, but also by providing an alternative H sink in the rumen (Johnson and Johnson, 1995). It may also be a consequence of defaunation of protozoa in the

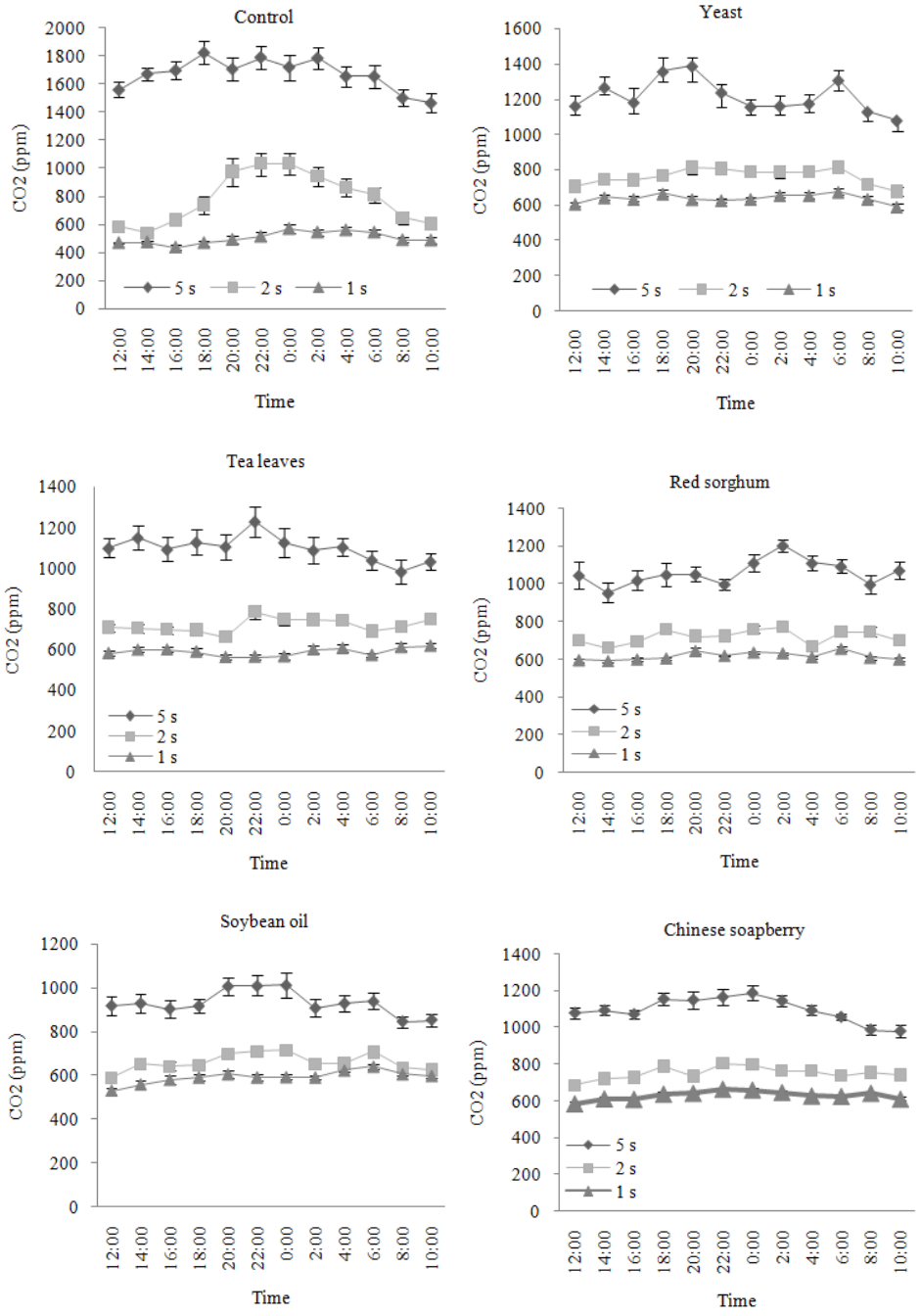


Fig. 2: Interaction between treatment and time on CO_2 concentration at 1, 2 and 5 s after opening the valve ($p>0.001$)

rumen (Hegarty, 1999) due to the toxic effect on methanogens associated with ciliate protozoa. Another reason might be hydrogenation, since ruminant bacteria use H to saturate the unsaturated fatty acids. This leads to the use of H for propionate formation instead of acetic acid. Our results are congruent with CH_4 reductions found in previous studies. For example, McGinn *et al*. (2004) reported a reduction of 21% with sunflower oil supplementation, while Machmüller and Kreuzer (1999) reported a reduction of 28% with coconut oil supplementation. Similarly, two studies by Jordan *et al*. (2006a, b) found that refined soybean oil supplementation was able to reduce CH_4 by 40 and 18%, respectively. However, those experiments were done *in vivo* and with higher supplementation levels.

It is known that *Camellia sinensis* is a saponin-tannin-rich plant. Saponins are secondary compounds found in many plants. It is believed that saponins kill or damage protozoa by forming complexes with sterols in the protozoal membrane surface (Sliwinski *et al*., 2002

and Wina *et al*., 2005) and change rumen fermentation patterns (Wang *et al*., 2000), so this may be an explanation for the reduced CH_4 concentration. Our results for tea leaf supplementation are in line with those of Hu *et al*. (2005), who reported a reduction of 26% in CH_4 production for *in vitro* conditions after supplementation of tea saponin at high doses (8 mg).

RS contains condensed tannins, which are secondary plant phenolic compounds that decrease degradation of nutrients in the rumen, which may then be degraded in the hindgut. Hindgut fermentation differs from ruminal fermentation in that there is lower CH_4 production per unit of fermented nutrients (Sliwinski *et al*., 2002; Min *et al*., 2003). Our results are in line with those of Hess *et al*. (2006), who reported a decrease in methanogenesis *in vitro* by using different plants rich in condensed tannins.

The decrease of CH_4 due to CS supplementation is related to its high saponin content. Results are similar to those of Argawal *et al*. (2006) who tested *Sapindus mukorossi* extracted with ethanol, water and methanol, reducing methanogenesis by 96, 20 and 22.7%, respectively. Our results also agree with those of Kamra *et al*. (2006) who reported complete inhibition of CH_4 production *in vitro* by supplementing ethanol extract from seed pulp. It is worth mentioning that the lower decrease of CH_4 in our experiment is due to the use of a byproduct from the soap industry instead of an extract. Thus, it could represent an important and feasible alternative for the diet of ruminants in countries where there is a huge amount of this byproduct, e.g., Taiwan.

Results for YS supplementation agree with Lynch and Martin (2002) and Mwenya *et al*. (2004), who found a significant decrease in CH_4 production when supplementing both live cells of *Saccharomyces cerevisiae* and yeast culture. Conversely, our results disagree with those of McGinn *et al*. (2004), where no CH_4 reduction was found when supplementing either Levucell SC or Procreatin-7 yeast to beef cattle. Therefore, there is no conclusive evidence of methanogenesis reduction when supplementing yeast, so it is important to find the variables that affect its effectiveness. Moreover, most of the research has been done *in vitro*, so *in vivo* experiments are also needed.

CO_2 is a product of anaerobic fermentation. Thus, at higher fermentation rates, higher CO_2 production is expected. In our research, both CH_4 and CO_2 concentration were higher in the control treatments. This leads to the assumption that the different supplementations used in this experiment change the pattern of fermentation. CO_2 may have captured H_2 to form propionate instead of producing CH_4, as explained by Takahashi (2006).

CONCLUSION

All feed supplementations tested in this experiment significantly decreased CH_4 and CO_2 concentration *in vitro* conditions. However, SBO was found to be the most efficient in reducing CH_4 and CO_2, followed by TL (*Camellia sinensis*). Thus, they may represent new alternatives to lessen GHG (CH_4 and CO_2) emissions *in vivo* and could be a course of action for the dairy cattle industry to mitigate global warming. The present *in vitro* results indicate that plant secondary compounds seem to have the potential to be used as feed additives for rumen manipulation to reduce CH_4 and CO_2 emissions. However, longer incubation time is advised for future studies to determine if the reduction of CH_4 and CO_2 is transient. Further research may extend the studied supplements to *in vivo* conditions, which will further help to validate the results.

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