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Combination Effect of Clove and Cinnamon Oil on *in Vitro* Rumen Gas and Methane Production

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Abstract

Clove and cinnamon oils were used for rumen manipulation in ruminant animal production. Their major component, eugenol and cinnamadehyde were proved to optimize rumen metabolism and microbial composition. The objective of this study was to evaluate combination effect of clove and cinnamon oil on rumen gas and methane production by using an in vitro rumen fermentation system. Three ruminal cannulated cows were used as donors of ruminal fluid and were individually penned indoors. The cattle were fed daily total mix ratio (tmr – 60% concentrate 40% alfalfa hay) and had free access to water at all time. Ruminal fluid for in vitro rumen fermentation system was prepared as in vitro hohenheim gas test method. The treatments were 1) control, 2) cinnamon oil 300 ppm, 3) clove oil 300 ppm and 4) combination of clove and cinnamon oil which were assigned in order to factorial design. The results indicated that insoluble gas fraction (b; 40.8, 46.91, 40.36 and 47.46 ml respectively) and potential of extent of gas production (|a|+ b; 43.01, 51.03, 42.96 and 50.45 ml respectively) were significantly different ($p < 0.01$) between treatments. Soluble gas fraction (a) and rate of gas production (c) were not different between treatments. Clove oil, cinnamon oil and their combinations affected methane production and both essential oil decreased methane production (18.15 ml/ g dm vs 11.80 ml/ g dm and 11.55 ml/g dm, $p<0.05$). However there were no additive or synergistic effect when they were used together (11.80 ml/g dm vs 11.55 ml/g dm). These results explained that some essential oils were used together as blend oil may not give additional beneficial effect.

Keywords: clove oil, in vitro rumen fermentation system, orange peel oil, rumen gas production, rumen methane production

Introduction

Public awareness of the potential health risk and environmental problem caused by the excessive use of in-feed antibiotics, growth hormones and some pharmaceutical food production lead to prohibition of some antibiotics since 1998 in EU member state. Some aromatic herbs and essential oils which have been used for animal health management may substitute the using of growth promoters such as antibiotics and hormones. Current interest in ruminant animal production industry is focused on trying to improve production efficiency and reduce environment effect (Methane emission and ammonia excretion) with using essential oils. Clove and cinnamon essential oils have been evaluated at some doses. Their major component, eugenol and cinnamaldehyde were proved to optimize rumen metabolism and microbial composition.

Some *in vitro* research of clove essential oils and euogenol oil from doses 3 to 5000 mg/L *in vitro* fermentation culture was reported had effect on all of rumen fermentation products (Busquets, 2005a) decreasing VFA concentration (Busquet, 2005b; Castillejos *et al*., 2006). *In vivo* experiments using used blend oils contain eugenol (Agolin ruminant) 0.5 g/day/cow which had no effected on dry matter intake and milk yield, but increased fat contain in milk (Santos, 2010). Cinnamon leaf essential oil (500 mg/l) containing 76% eugenol decreased N-ammonia concentration, molar of branched-chain VFA (Fraser et al., 2007), acetate but increased propionate and butyrate at doses 312 mg/l (Busquet *et al*., 2005b). Cinnamon leaf oil (250 mg/l) also reduced methanogenesis activity of rumen bacteria and methane concentration in the fermentation gases, without altering total VFA (Chaves *et al*., 2008). *In vivo* studies reported that supplementation of cinnamaldehyde (200 mg/kg of dry matter) had no effect on dry matter intake, gain, feed efficiency, carcass characteristics, meat quality and rumen N-ammonia concentration in growing lamb fed barley or corn-based diets (Chaves, 2008).

The combination between essential oils or their components may result in additive and/or synergistic effects that may enhance efficiency of rumen microbial fermentation and nutrient utilization in ruminants. Thus, this research was evaluated the effects of clove (eugenol), cinnamon(cinnamaldehyde) and their combinations in the *in vitro* fermentation system.

Materials and Methods

Three ruminal cannulated cows were used as donors of ruminal fluid and were individually penned indoors. The cow was fed daily total mix ratio (TMR – 60% concentrate 40% alfalfa hay) twice at 08.00 and 14.00 h and had free access to water at all time.

Clove (CO) and cinnamon (CIN) essential oils were extracted using methanol

from clove bud and cinnamon bark. The chemical composition of clove and cinnamon essential oil samples indicated that eugenol contain in clove oil is 97.26% and cinnamaldehyde contain in cinnamon oil is 17.79%. The TMR that was used for ruminal animal donor, was also used as a substrate for *in vitro* rumen fermentation system. The TMR contained protein 18.81%, ADICP 9.22 %CP, NFE 49.87%, crude fibre 19.77%, NDF 38.22 and ADF 29.24%

The treatments were 1) control, 2) Cinnamon oil (CIN) 300 ppm, 3) Clove oil (CO) 300 ppm and 4) Clove oil (CO) 300 ppm + Cinnamon Oil (CIN) 300 ppm. Addition of CO, CIN and their combination were evaluated by *in vitro* Hohenheim Gas Test (HGT). The method of the HGT system is described in detail by Menke and Steingass (1979).

Anaerobic techniques were used in all procedures during the rumen fluid transfer and incubation period. Rumen fluid was collected from rumen-fistulated cows before morning feeding after 2 weeks feed adaptation. Filtered Rumen fluid was added to buffer medium in proportion 1 : 2 v/v, keep with carbon dioxide flushing for 10 -15 minute before starting to fill up the syringes. CO and CIN oils 300 ppm added to mixed rumen fluid and buffer medium as a treatments of this experiment. Through the inlet of the HGT glass syringes which substrate is placed inside it , 30 ml incubation mixed medium (rumen fluid, buffer medium and CO,CIN or combination CO-CIN) then dispensed into the pre-heated HGT glass syringe (39 ^{o}C) with the help of a semi-automatic pipette. The incubation was conducted for 96 hours inside the modified water bath (39 ^{o}C). Buffer medium containing some solution which is described by Menke and Steingass (1979).

Gas production data was collected at 3, 6 , 9, 12, 24, 48, 72, 96 hours. After 6 h of incubation methane gas was measured using a catharometer methane sensor OLC20 (Oldham ®, USA). The methane sensor was calibrated at 0–100% methane at gas produce at the time, and was calibrated again using pure methane gas (99,7%). Cumulative gas production data were fitted to the model of Orskov and Mcdonald (1979):

$$y = a + b(1-exp^{-ct})$$

where : a - gas production from immediately soluble fraction (ml). b- gas production from the insoluble fraction (ml). [a + b] – potential gas production (ml). c – gas production rate constant for the insoluble fraction (ml/h). t – incubation time (h). y – gas produce at time t (ml)

Estimation of metabolic energy of TMR was calculated by equations from Alderman (1985) using proximate values of TMR and from Boguhn et al (2003) using gas production value. The predicted value of ME of TMR for this experiment is 2.70 Mcal/Kg.

A two (CO0-CO300 ppm) by two (CIN0-CIN300) factorial arrangement in a completely randomized designed was used to compare gas production, gas produc-

tion kinetics and methane production using the General Linear model (GLM) of the SAS. The significance of differences between individual means wre determined using Duncan's multiple comparation test.

Results and Discussion

Soluble gas fraction (a) and rate of gas production (c) were not different between treatments. These data suggested that a lag phase due to delay in microbial colonization of the TMR may occur in the same time of incubation. The fermentation TMR by using CO, CIN and their combination had significant effect on insoluble gas fraction (b), potential of extent of gas production (|a|+b), gas production after 96 h incubation and methane gas production after 6 h incubation (Table 1). The value for a intercept for all treatments was same .

The addition of CIN 300 ppm and combination between CO and CIN resulted in higher value of gas volume at asymptote (b) than control treatment but athe addition

Table 1. Methane production at 6 hour incubation, characteristic and cumulative gas volume production throughout 96 hour of rumen incubation with TMR, CO, CIN, and combination between CO-CIN at 300 ppm

Gas Parameter	CO-0		CO300		SE	P < 0.05		
	CIN-0	CIN300	CIN-0	CIN300		CO	CIN	CO*CIN
Gas characters :								
a	-2.22	-4.12	-2.60	-2.99	0.66	0.59	0.11	0.28
b	40.80^{b}	46.91^{a}	40.36^{b}	47.46^{a}	1.17	0.97	0.00	0.68
c	0.08	0.08	0.09	0.07	0.01	0.36	0.36	0.11
[a] + b	43.01^{b}	51.03^{a}	42.96^{b}	50.45^{a}	1.97	0.27	0.03	0.28
Gas Production :								
6 h	12.50^{b}	14.21^{a}	12.86^{ab}	12.53^{b}	0.44	0.16	0.15	0.04
24 h	31.28^{b}	36.17^{a}	30.75^{b}	34.59^{a}	0.59	0.1	0.00	0.39
48 h	37.31^{b}	41.69^{a}	37.27^{b}	42.13^{a}	0.97	0.84	0.00	0.81
96 h	38.74^{b}	42.87^{a}	37.70^{b}	44.56^{a}	1.76	0.85	0.01	0.45
pH	6.78	6.76	6.76	6.74	0.01	0.19	0.14	1.00
ME (MCal/ KgDM)*	2.25^{b}	2.30^{a}	2.25^{b}	2.29^{a}	0.01	0.10	0.00	0.39
Methane %	26.55^{a}	21.11^{b}	23.18^{b}	18.86^{c}	0.79	0.01	0.06	0.09
(ml/g DM)	18.15^{a}	11.80^{c}	14.90^{b}	11.55^{c}	0.01	0.02	0.07	0.08
MR (%)		34.99	17.91	36.36				

CO= Clove oils, CIN = Cinnamon Oils, MR= methane reduction, ME*= predicted by equation from Boguhn, J. *et al.* (2003), Same letter at the same row indicate to no difference bettween treatment (P>0.05).

of CO 300 ppm had same value with control. The gas volume at asymptote (b) represented the fermentation of the insoluble fraction. This result might indicated that the addition of CO at 300 ppm had no negative effect on digestibility of insoluble fraction of TMR, because CO at level 0.25 ml and 0.50 ml could inhibit CMCase, xylanase and acetylesterase activity (Patra, 2010). The addition of CIN 300 ppm and combination between CO and CIN could increased digestibility insoluble fraction of TMR due to high value of gas production at asymptote (b) and total gas production at 96 h after incubation. There is no interaction effect between CO and CIN on gas production at asymptote (b) value ($P > 0.05$).

The Addition of CIN 300 ppm and combination between CO 300 ppm and CIN 300 ppm had potential extent of gas production value higher than control, but the addition of CO had same value with control. These results indicated that the addition CIN and combination CO and CIN affect digestibility of insoluble fraction of TMR. Gas production 24 h after incubation increased with the addition of CIN and combination CO-CIN which it might efficiency of energy using from TMR. Addition of CIN 300 ppm and combination CO and CIN had predicted metabolism energy after 24 h of incubation higher than control. Menke et al (1988) suggested that gas volume at 24 h after incubation has a relationship with metabolizable energy in feedstuff.

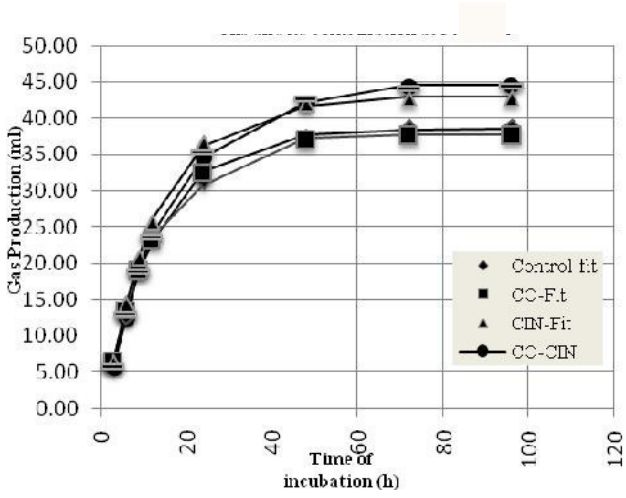

Figure 1. Cummulative gas volume estimated by $Y = a + b\,[1-\exp(-ct)]$ throughout 96 hour. substrat is TMR added with CO, CIN and its combination at 300 ppm

Available energy of TMR affected on gas production and methane emission from rumen fermentation. Addition of CIN 300 ppm, CO 300 ppm and combination CO 300 ppm and CIN 300 ppm reduced methane production after 6 h incubation from control (34.99%, 17.91% and 36.36%, respectively). Methane reduction value of addition CIN 300 ppm had twice value from addition CO 300 ppm, but addition combination of CO and CIN had same methane reduction value with the addition

CIN 300 ppm. It indicated that there was no synergy effect between CO 300 ppm and CIN 300 ppm at in vitro gas production test.

Conclusions

The addition of cinnamon 300 ppm, clove 300 ppm and their combination affected on methane reduction. Methane reduction value of addition cinnamon was twice higher than addition of clove, but there had no synergy effect of methane reduction value of addition combination between cinnamon and clove. Methane gas reduction of addition clove, cinnamon and their combination due to efficiency of energy available in TMR from insoluble fraction of TMR.

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